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SIMULATION OF LILY ROSETTE SYMPTOMS BY FEEDING INJURY OF THE FOXGLOVE APHID

FLOYD F. SMITH[1] AND PHILIP BRIERLEY[2]

(Accepted for publication May 10, 1948)

Lily rosette, or yellow flat,[3] is one of the most destructive virus diseases of Easter lily (*Lilium longiflorum* Thunb.) and is also transmissible to other species of *Lilium*.[4] This is one of the most difficult diseases of lily to diagnose, for the symptoms of other diseases and injuries may resemble more or less closely the rosetting and curling effects of the rosette disease. We therefore consider that the only positive means of identifying this disease at present is the melon aphid (*Aphis gossypii* Glover), specific vector of the rosette virus. By this means this disease has been identified from field plantings in Florida twice and in Texas once. The rosette disease has not been confirmed from other field samples submitted. It is certain that similar diseases have been reported as rosette more than once. The most common symptoms that bear a strong resemblance to rosette result from cold injury, waterlogging, and diseases of the bulbs and roots, such as fusarium rot.[5] A less common but very similar symptom induced by the feeding of the foxglove aphid (*Myzus convolvuli* (Kltb.)) is described below.

Myzus convolvuli was collected on Easter lilies in a Long Island greenhouse in November, 1939, and again in 1945. Some curling of the infested leaves suggestive of rosette disease was present at both times. Aphids from the second collection were placed on an Easter lily seedling infected with rosette virus from November 26 to December 5, 1945, and then colonized on five healthy Easter lilies for 6 days. After removal of the aphids the plants were observed for 3 months, but no symptoms of rosette disease developed. This aphid is therefore eliminated as a possible vector of the lily rosette virus.

On December 11, 1945, twenty specimens of *Myzus convolvuli* were transferred to three additional healthy Easter lilies and allowed to feed and reproduce until January 18, 1946, when they were removed or destroyed. At this time all three plants showed marked downward curling of the young leaves on which the aphids had been feeding, and also an irregular yellow mottling of the old leaves (Fig. 1, *A*). These feeding effects had a very close resemblance to the symptoms of rosette disease (Fig. 2), and also suggested the presence of lily mottle disease. However, further growth of these

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[3] Ogilvie, L. A. A transmissible virus disease of the Easter lily. Ann. Appl. Biol. 15: 540–562. 1928.

[4] Brierley, Philip, and Floyd F. Smith. Additional species of *Lilium* susceptible to lily rosette virus. Phytopath. 35: 129–131. 1945.

[5] Brierley, Philip, Floyd F. Smith, and F. P. McWhorter. Diseases of Easter lilies important in domestic production. Florists' Rev. 93 (2411): 21–23, 94. 1944.

FIG. 1. Easter lily showing effects of feeding by foxglove aphids: A, Stunting, curling, and mottling in young growth; B, Persisting old injury and normal new growth in same plant five weeks after removal of aphids.

plants after removal of the aphids was normal (Fig. 1, *B*), the curling persisting at the zone of injury only.

Gadd and Loos[6] described a disease condition in *Lilium longiflorum* in Ceylon and also the symptoms of leaf curling and chlorotic spotting that developed after healthy lilies were colonized with green aphids of an unidentified species. They also reported the development of chlorotic spots

FIG. 2. Easter lily infected with rosette disease by viruliferous *Aphis gossypii*.

[6] Gadd, C. H., and C. A. Loos. Lily mosaic. Trop. Agr. (Ceylon) 94: 160–167. 1940.

on cucumber, tomato, and turnip in about 5 days after the transfer of the same aphid from lily. They concluded that the virus involved was that of cucumber mosaic, although the symptoms were confined to the leaves fed upon by the aphids. The symptoms they illustrated were not typical of mosaic patterns and resembled the feeding injury by the foxglove aphid in our experiments. In response to our request Mr. Gadd sent us specimens of his green aphid, and P. W. Mason confirmed our identification of these specimens as being typical *Myzus convolvuli*. Mr. Gadd also indicated that the diseases of Easter lily in Ceylon were apparently a complex of viruses which included both necrotic fleck and rosette.

In the authors' experiments with lily viruses extending over several years, *Myzus convolvuli* failed to transmit lily mottle (0/29),[7] cucumber mosaic (0/8), lily symptomless virus (0/37), and lily rosette virus (0/8). Chlorotic spots or leaf curling, or both, followed in 5 or 7 days after this aphid was colonized on several of its many hosts, including African violet (*Saintpaulia ionantha* Wendl.), geranium (*Pelargonium hortorum* Bailey), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), turnip (*Brassica rapa* L.), and lily. The symptomatic period of the lily viruses was 10 days or longer when transmitted by their known aphid vectors.

It is clear that *Myzus convolvuli* is not a vector of either lily rosette virus or lily mottle virus, although its feeding injuries simulate the symptoms of both virus diseases.

This species is encountered only occasionally on lilies in greenhouses, but establishes persistent colonies where it occurs. Fairly large numbers on rapidly expanding young growth are needed to induce the injury simulating lily rosette, but it seems likely that such injury will be observed occasionally and may be misinterpreted by observers who are not forewarned.

AGRICULTURAL RESEARCH CENTER,
BELTSVILLE, MARYLAND.

[7] Ratio of plants infected to plants exposed.

NUCLEAR INCLUSIONS PRODUCED BY A STRAIN OF TOBACCO MOSAIC VIRUS[1]

M. W. Woods[2] and Richard V. Eck[3]

(Accepted for publication May 10, 1948)

INTRODUCTION

Although nuclear inclusions have been described in about 30 different virus diseases of animals, they have been reported much less frequently in plants. Hoggan mentioned finding square or oblong plates of undetermined nature in nuclei of solanaceous plants infected with tobacco mosaic virus (4). Kassanis (6) and Bawden and Kassanis (2) have reported protein crystals in the nuclei of solanaceous plants infected with severe etch or mild etch virus. McWhorter (8, 9) observed crystalline inclusions in both nuclei and nucleoli of leguminous plants infected with either Pisum virus 2 or Phaseolus virus 2. So far as we are aware, these constitute the only plant virus diseases in which nuclear inclusions have been described. These authors reported that the nuclear inclusions associated with the above mentioned legume and etch viruses were structurally rather stable, could be micromanipulated from the nuclei, and were not soluble under physiological conditions. Such inclusions do not occur in the nuclei of healthy plants. Although the inclusions induced by etch virus were very similar to the nucleoli in their staining reaction, Sheffield (10) has been able to demonstrate differences. She points out that in general these plant nuclear inclusions, which show protein color reactions, have many properties in common with the nuclear protein inclusions characteristic of the polyhedral diseases of *Lepidoptera*.

The present paper reports nuclear inclusions of types that so far as we can find have not been described before, although Hoggan (4) may have observed one of these types.

MATERIALS AND METHODS

Virus. The strain of tobacco mosaic virus (*Marmor tabaci* Holmes) which induces the formation of nuclear inclusions (1C) has been described elsewhere (5). For purposes of comparison, a strain of ordinary "green mosaic" (2A) and a "yellow mosaic" mutant (2A–1) were used.

Plant Material. Test plants included *Nicotiana tabacum* L. var. Turkish, *N. tabacum* L. var. Maryland Medium Broadleaf, *N. paniculata* L., *N. glutinosa* L., *Capsicum annum* L. var. California Wonder, and *Lycopersicon esculentum* Mill. var. Rutgers. Leaf tissue for histological study was prepared by infiltrating the intercellular spaces with a complete nu-

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trient salt solution containing 5 per cent sucrose. The tissue was immersed in this solution in a flask, and suction applied intermittently. Then sections 4–5 cells thick were cut with a very sharp razor. Tissues so prepared may be kept alive for several days on the microscope slide if the solution is changed to provide oxygen.

SYMPTOMS

The "nuclear strain," 1C, induces a diffuse yellow spotting on inoculated leaves of *Nicotiana tabacum,* followed by a bright yellow systemic mottling similar to aucuba mosaic. Similar symptoms are produced on *N. paniculata, Lycopersicon esculentum,* and *Capsicum annum.* Localizing necrotic lesions are produced on *N. glutinosa.* Nuclear inclusions have been observed in all of these species, except *N. glutinosa,* when infected with virus 1C.

PROPERTIES OF THE VIRUS

With respect to thermal inactivation and resistance to desiccation, virus 1C behaved in a manner typical of tobacco mosaic virus. Material dried one year or heated to 80° C. for ten minutes induced typical symptoms and nuclear inclusions.

Physical Properties. With respect to size and shape, the particles of all three strains are typical for tobacco mosaic (5). Virus 1C, however, has a somewhat higher isoelectric point and can be separated from mixtures with 2A by cataphoresis (5).

INTRACELLULAR INCLUSIONS

Three types of inclusion bodies have been observed in affected cells. (a) Crystalline or fibrous proteinaceous inclusions in the cytoplasm, (b) loosely aggregated amorphous proteinaceous precipitates in the cytoplasm, the "X-body" type, and (c) inclusions in the nucleus which are apparently identical or very similar to the types formed in the cytoplasm. Inclusions have not been found in nucleoli.

The crystalline or fibrous inclusions are very unstable and go into solution when the cell is mechanically injured. The amorphous precipitates are not destroyed by such treatment. The unstable type of inclusions in both cytoplasm and nucleus present a wide variety of forms (Fig. 1 and 2), although within lesions of the same age on the same leaf considerable uniformity exists.

The number of both nuclear and cytoplasmic inclusions reaches a maximum in from 10 to 20 days after infection of the cells. Beyond this time these inclusions have been observed less frequently. Similar behavior of cytoplasmic inclusions in ordinary tobacco mosaic has been described (7).

Nuclear inclusions of the types described here were never observed in non-infected tissues. It is noteworthy that in spite of the polymorphism of the inclusions there was a marked tendency for them to take the same form in the nucleus and cytoplasm of the same cell (Fig. 1). This would

indicate that the conditions affecting the orientation of particles as the inclusions are formed are similar in the cytoplasm and nucleus of a cell. An exception was the rarely observed ring-shaped inclusion (Fig. 2, E). This type was found in the nuclei only. They strongly resemble the rings and figures of eight, described by Suchkov (11) in the cytoplasm of mosaic-infected gramineae.

The cytoplasmic inclusions induced by strain 1C, and those formed by typical green and yellow mosaic (2A and 2A–1) seem to have many properties in common. On treatment with HCl at pH 1.3 they all break down, frequently with formation of needles. If treated with saturated ammonium sulfate they do not break down when the cell is ruptured or treated with certain reagents. Such salt treatment makes possible various tests.

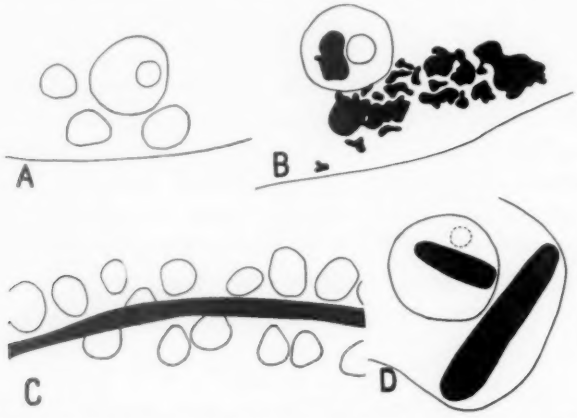

FIG. 1. Nuclear and cytoplasmic inclusions in living leaf cells of *Nicotinia tabacum* L. var. Turkish infected with tobacco mosaic virus strain 1C. In all drawings viroplastic inclusions appear as solid black. A. Nucleus and plastids of non-infected cell. B. Cell with amorphous trypan blue-positive nuclear and cytoplasmic inclusions. C. Portion of long fibrous cytoplasmic inclusion lying among plastids. D. Elongated plate-like nuclear and cytoplasmic inclusions in one cell. All figures represent line drawings over original photomicrographs. 785×. A striate structure not shown in the drawings was visible in the fibrous inclusions.

The inclusions then give a red coloration with Millon's reagent, and an orange yellow color when treated with concentrated HNO_3 and NH_4OH. These inclusions are therefore probably proteinaceous. Trypan blue (0.05 per cent) intensely stains the non-crystalline inclusions ("X-bodies") induced by all three virus strains whereas the crystalline or fibrous inclusions do not so stain. The nuclear inclusions induced by virus 1C behave in all these respects like the cytoplasmic inclusions.

Killed Nuclei. Nuclei of cells infected with virus 1C and killed in sectioning, often presented a very characteristic appearance not observed in cells infected with the other viruses. In many instances all of the inclusion material had gone into solution leaving a very large clear space (usually occupying most of the nuclear volume) with the visible structural material of the nucleus, including the nucleolus, surrounding it. The nucleoli were

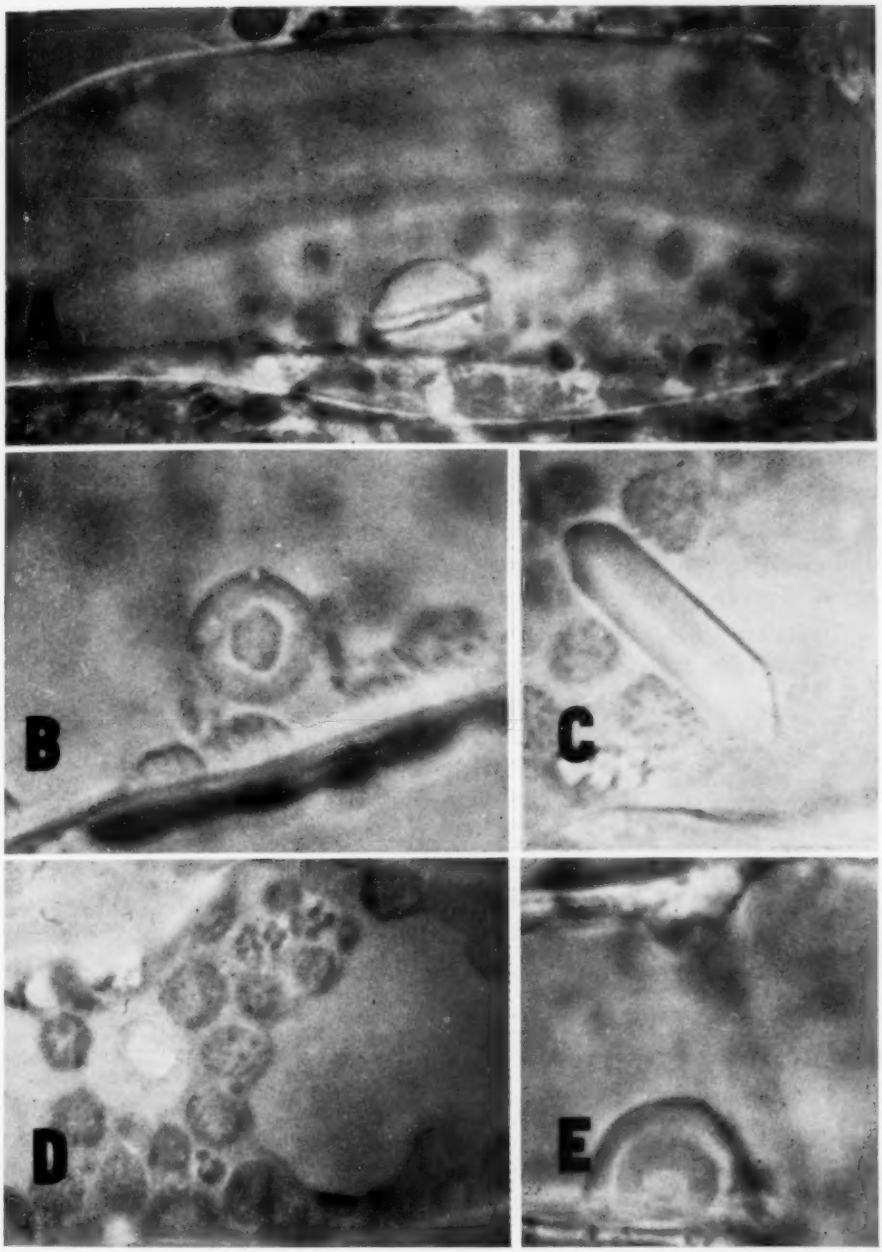

FIG. 2. Photomicrographs of living Turkish tobacco mesophyll cells containing nuclear and cytoplasmic inclusions. A. Fibrous inclusions. B. Platelike inclu.ion surrounded by clear space in nucleus. C. Platelike inclusion in cytoplasm. D. Block-like nuclear inclusion. E. Ring-shaped inclu.ion surrounding nucleolus. All pictures 1535×. A line drawing of A appears in figure 1.

apparently not changed structurally. Treatment of such nuclei with saturated ammonium sulfate generally resulted in precipitation in the central clear space of masses of closely packed parallel needles. These needles gave positive Millon's and xanthoproteic reactions. Some damaged nuclei (not treated with ammonium sulfate) contained within the central clear space extensive needle- or spike-like crystalline deposits, usually much larger than those formed following treatment with ammonium sulfate. Such forms were not observed in undamaged nuclei.

SUMMARY AND CONCLUSIONS

All of the evidence to date shows that the nuclear inclusions induced by tobacco mosaic virus strain 1C are similar or identical to those occurring in the cytoplasm of the same cells. The nuclear and cytoplasmic inclusions induced by this strain reacted in all our tests like the various proteinaceous cytoplasmic inclusions found in cells infected with ordinary types. As Beale (3) and Bawden (1) have pointed out, the crystalline inclusions found in these infections are probably composed at least in part of virus. The present data suggest, therefore, that a strain of tobacco mosaic virus exists which is capable of invading nuclei of cells.

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BLOSSOM AND SPUR BLIGHT (SCLEROTINIA LAXA) OF SOUR CHERRY

E. C. Calavan and G. W. Keitt[1]

(Accepted for publication May 17, 1948)

Blossom and spur blight of sour cherry (*Prunus cerasus* L.), incited by *Sclerotinia laxa* Ader. and Ruhl., was discovered in Baileys Harbor Township of Door County, Wisconsin in 1941 (21). Subsequently, the fungus was found in the Sturgeon Bay cherry district, where several local outbreaks occurred. Although its natural occurrence in the eastern half of North America was established only recently (21), *Sclerotinia laxa* has been a serious pest for many years in most other humid stone-fruit regions of the world, with the exception of Australia and New Zealand (14, 16, 35, 42, 44).

The time and manner of introduction of *Sclerotinia laxa* into North America are unknown. The first detailed cultural studies of American isolates were made by Posey (31, 32) in 1913 in Oregon. At that time spur blight was already established in most humid stone-fruit areas in British Columbia and the Pacific Coast States and probably had been present in central California since about 1900 (6, 16). In the Pacific Coast States *S. laxa* has become the major brown-rot organism in some localities (3, 16, 23). East of the Rocky Mountains it has been reported only from Wisconsin.

Sclerotinia laxa has been studied extensively in Europe and western North America, but no comprehensive work concerning its development and satisfactory control on sour cherry under conditions closely approximating those of Wisconsin has been reported. This paper presents the results of experimental studies from 1941 to 1945 concerning this pathogen and its control on sour cherry in Wisconsin. A preliminary report has been published (9).

SYMPTOMS

The first symptoms on sour cherry appear as brown lesions on the petals or as a browning of the stigma, style, or stamens. In stigma infection the darkened area spreads down the style (Fig. 1, A) to the ovary, from which it extends outward to other flower parts and downward through the pedicel. From a diseased pedicel, infection may extend to the supporting twig or branch. With stamen infection, browning usually appears at the tip of the filament but soon progresses to the rim of the floral cup, where a small, light-brown lesion is formed at the juncture of the stamen and the rim (Fig. 1, B). This spot enlarges into a dark-brown, "U"-shaped area on the side of the floral cup (Fig. 1, C). From the floral cup the browning extends through the receptacle and pedicel. Occasionally infection may reach

[1] Grateful acknowledgments are made to J. Duain Moore for participation in the experiments on control, to Eugene Herrling for preparation of the illustrations, and to E. E. Wilson, C. E. Owens, S. M. Zeller, and Folke Johnson for collections of *S. laxa*.

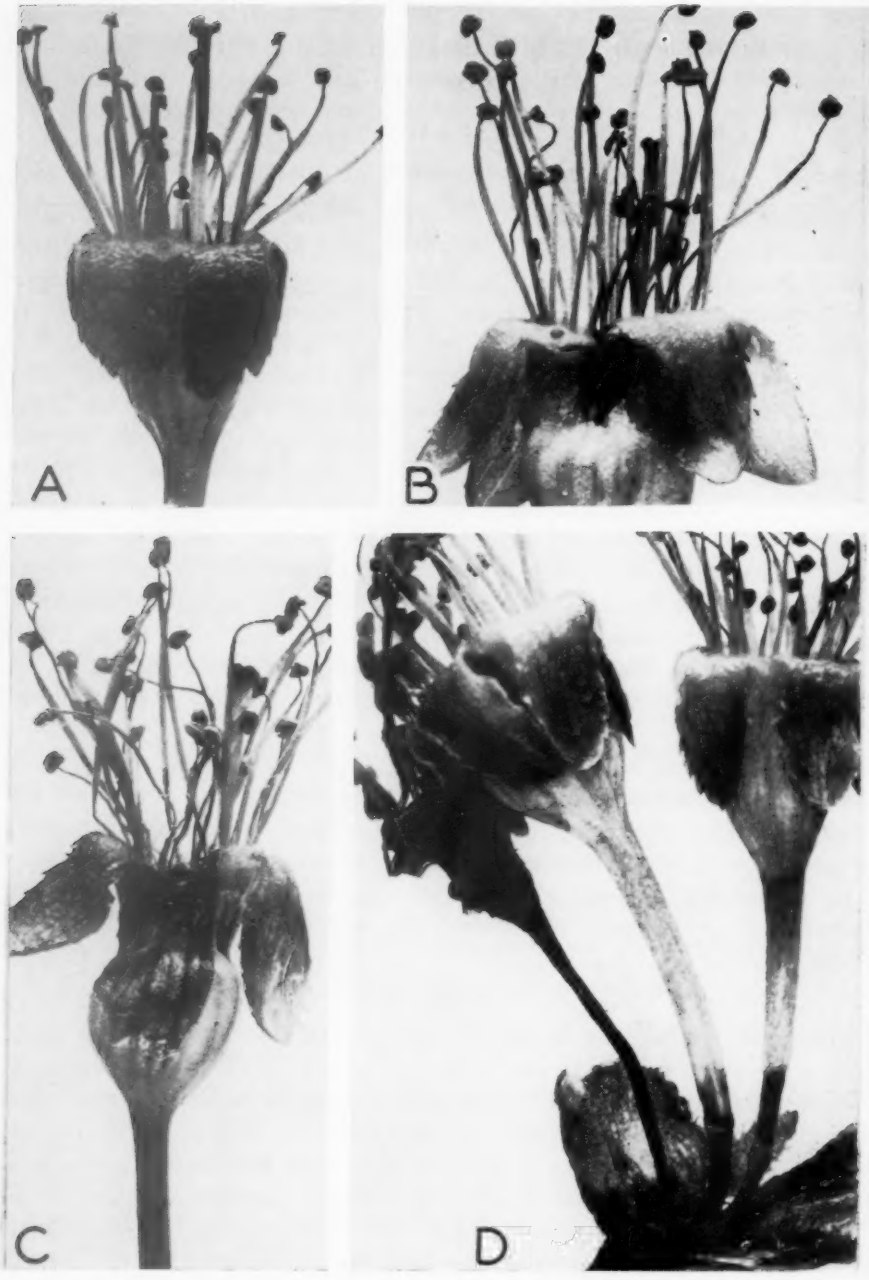

FIG. 1. Sour cherry blossoms infected by *Sclerotinia laxa*. A. Lesion on the style following stigma infection. B. Small lesion on the floral cup following stamen infection. C. Advanced lesion on the floral cup. D. Diseased blossom cluster showing spread of infection from the base of one pedicel into two others.

the rim of the floral cup from a diseased petal and produce symptoms resembling those resulting from stamen infection.

The fungus spreads readily from the base of a pedicel to surrounding bracts and to other pedicels of the cluster, and thus it rots the pedicels of flowers which previously may have escaped infection (Fig. 1, D).

Wilting of blossom clusters occurs within 4 to 15 days after infection. During moist weather such clusters may bear a loose layer of gray, sporulating hyphae or occasional small sporodochia. Leaves on diseased spurs may wilt at any time from 10 days to 6 weeks after blossom infection. Pendant clusters of shriveled leaves and blossoms (Fig 2, E, F) constitute the most conspicuous manifestation of this disease.

Frequently the fungus grows from killed spurs into larger branches, where its invasion often leads to the formation of elliptical cankers (Fig. 2, G). When the attack is especially severe, branches may be girdled and the distal portions killed within a few weeks (Fig. 2, E). Gum pockets commonly are present near the margins of young cankers, and masses of clear to amber-colored gum often exude from diseased spurs and branches.

Gray sporodochial tufts (Fig. 2, A, B, C), usually less than 1.0 mm. high and from 0.5 to 3.0 mm. in diameter, form in the spring from shortly before budbreak (in Wisconsin) until shortly after bloom. Sporodochia may develop anywhere on the dead twigs or adherent parts but are especially abundant between bud scales, on the bracts, and in the floral cups of overwintered blossoms.

Although *Sclerotinia laxa* is most important as a blossom- and twig-blighting organism, it also is able to attack both green and ripe fruits, upon which it induces symptoms very similar to those of American brown rot incited by *Sclerotinia fructicola* (Wint.) Rhem. Decaying fruits darken and may be covered by numerous spore tufts during moist weather.

Leaf infection, except by direct contact of leaves with diseased material, is rare. However, it was obtained experimentally in the greenhouse following inoculation by conidial suspensions. Lesions appeared first as small, chlorotic spots which soon became gray to brown. Under very moist conditions the necrotic areas enlarged rapidly (Fig. 2, D) and occasionally extended down the petiole to the shoot, as reported on sweet cherry by Maier and Mittman-Maier (26).

The symptoms described above agree in many respects with those reported for European brown rot of stone fruits on various hosts in Europe and western North America (10, 18, 24, 44).

THE CAUSAL ORGANISM

The taxonomy, synonymy, and history of *Sclerotinia laxa* Ader. and Ruhl. and closely related species have been reviewed by many authors (7, 13, 15, 17, 27, 35, 42) since the first brief description of *Torula fructigena*, from specimens possibly containing both *S. laxa* and *S. fructigena*, was published by Persoon (30) in 1796. Some uncertainty remains, however, regarding the

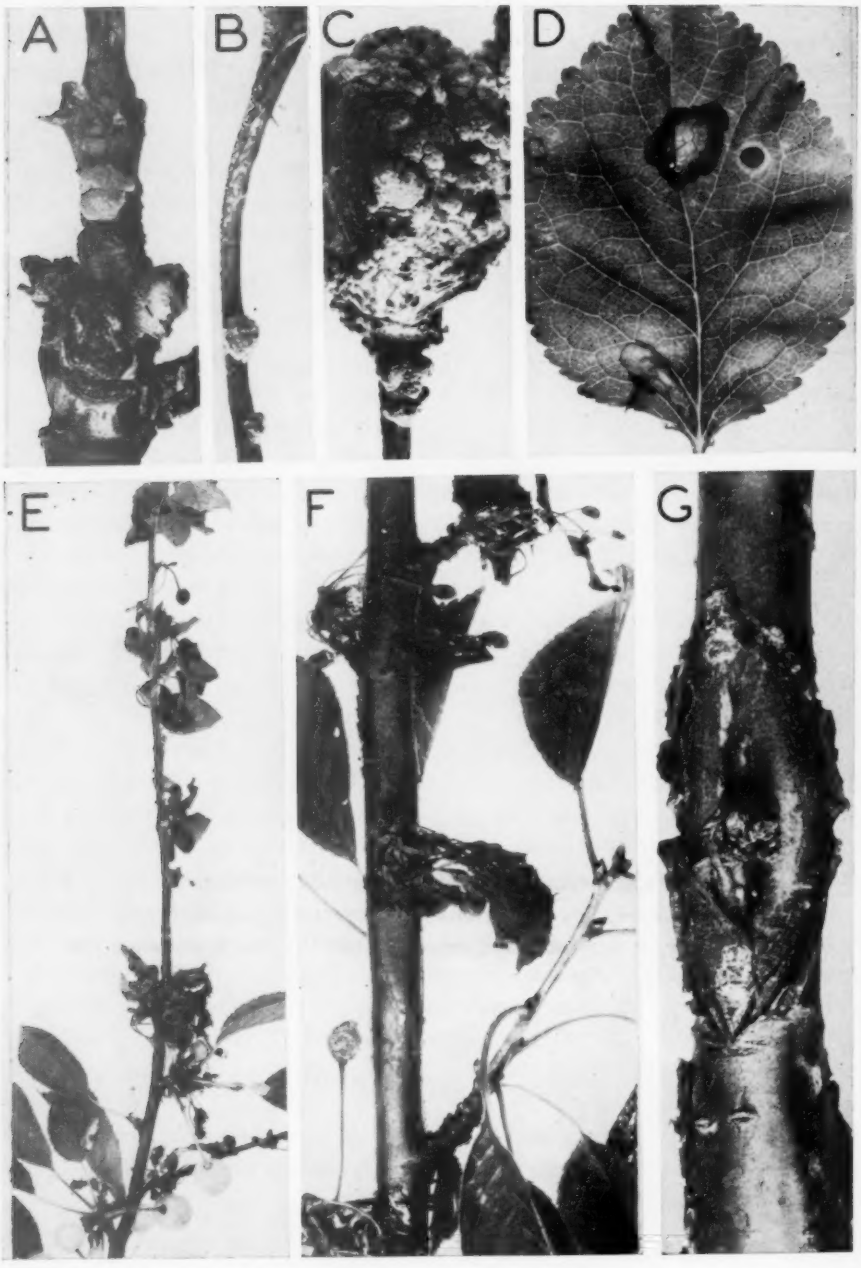

FIG. 2. *Sclerotinia laxa* on sour cherry. A–C. Sporodochia on overwintered dead parts. D. Leaf lesions. E. Blighted terminal shoot. F. Blighted spurs. G. Branch canker.

ultimate disposition of certain questions concerning the correct binomial for the supposed *S. laxa* of North America. Among the phenomena which merit further investigation are the ability of an isolate of *S. fructicola* to assume the colony characters of *S. laxa* under certain conditions (Fig. 3), the existence of numerous variants with colony characters *in vitro* intermediate between those typical of *S. laxa* and *S. fructicola* (Fig. 4, G-J), and the apparent absence of apothecia of *S. laxa* in North America. Possibilities exist that North American isolates herein designated as *S. laxa* may be variants of another species (possibly *S. fructicola*), hybrids, heterocaryons, or a species of *Monilia* having no perfect stage.

Reference of the fungus inciting blossom and spur blight of sour cherry in Wisconsin to *Sclerotinia laxa* Ader. and Ruhl. is based on comparisons of

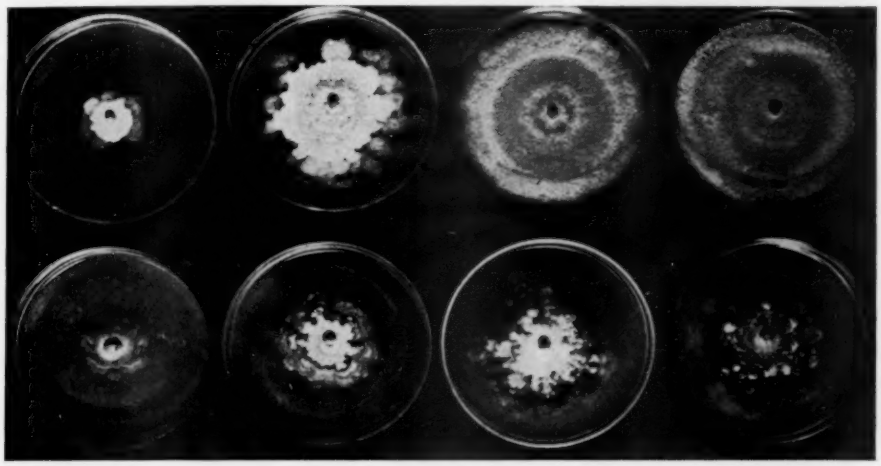

FIG. 3. Colonies of *Sclerotinia fructicola* (upper row) and *S. laxa* (lower row) on potato-dextrose agar 33 days at the following temperatures: left to right; 4°, 8°, 12°, and 16° C. Cultures in the upper row are from plum at Madison, Wisconsin; those in the lower row, from sour cherry at Baileys Harbor. Note lobing and zonation of the *S. fructicola* cultures at 4° and 8° C.

Wisconsin isolates with isolates of the organism widely accepted as *S. laxa* obtained from California, Oregon, and Washington and on numerous descriptions and illustrations of the fungus in American and European literature (4, 10, 13, 16, 28, 31, 41, 43). This identification of the fungus is regarded as tentative pending possible discovery of its perfect stage and the development of a better basis for differentiating *Sclerotinia* spp. on stone fruits.

Colony characters. On potato-dextrose agar and on many other artificial media, *Sclerotinia laxa* characteristically formed rounded lobes at the margin of the colony (Fig. 3). However, different isolates varied greatly in the number and width of lobes produced (Fig. 4), and some isolates from almond (California) formed only a few lobes.

Concentric zonation is another important colony character of all Wiscon-

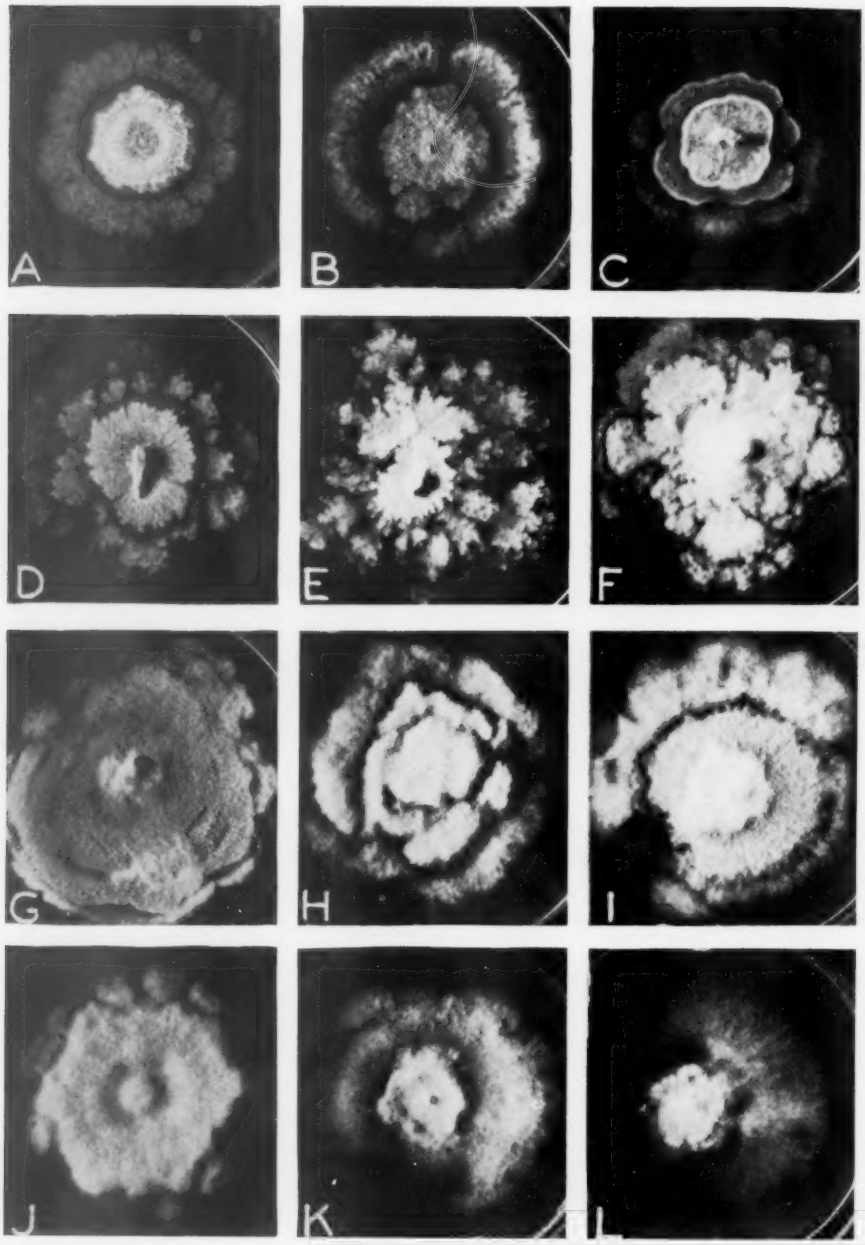

FIG. 4. Colony forms of *Sclerotinia laxa* on potato-dextrose agar 20 days at 16° C. A–C. Nos. 71, 67, and 93, from immature fruits of *Prunus cerasus*, Baileys Harbor, Wisconsin. D. No. 1, from spur of *P. cerasus*, Baileys Harbor. E. No. 86, from spur of *P. pennsylvanica*, Baileys Harbor. F. No. 51, from twig of *Chaenomeles japonica*, Corvallis, Oregon. G. No. 68, same source as A–C. H, I. Nos. 35 and 36, from blossoms of *P. Amygdalus*, California. J. No. 50, from mummified fruit of *P. domestica*, Corvallis, Oregon. K, L. Nos. 42 and 41, from twigs of *P. Amygdalus*, California.

sin isolates and most others examined. Zones apparently are produced by the periodic interruption of radial growth in favor of a thickening of the mycelial mat. After each interruption radial growth is resumed, usually from points other than the apices of the lobes, but the new growth remains very thin as long as radial growth continues. Lobing and zonation in artificial cultures of *Sclerotinia laxa* have been discussed extensively by Killian (24).

The color of cultures of *Sclerotinia laxa* on potato-dextrose agar or on other media may vary with different isolates. Young cultures are usually white or gray until the first interruption of their radial growth, which normally occurs when they are 20 to 40 mm. in diameter. At about this time the sub-surface mycelium begins to darken, particularly at the place of seeding and near the margins of the lobes. As the colony ages this mycelium often becomes black or greenish black, though in a few isolates it turns yellow or reddish brown. The aerial hyphae usually retain their light color longer than those in contact with the substratum.

Sporulation in culture. The scarcity of *Sclerotinia laxa* conidia in culture has been recorded by Berkhout (5), Ezekiel (13), and many others. It has constituted a serious problem to workers desiring to make large-scale inoculations by means of spore suspensions.

Most isolates of *Sclerotinia laxa* produced few or no conidia on potato-dextrose agar and various other artificial substrata at moderate temperatures. Therefore, various methods were tried to obtain sufficient conidia for inoculation experiments. Petri-dish cultures on nutrient, nutrient-dextrose, corn-meal, malt, oat, potato-dextrose, and prune agars, prepared according to the formulae in Riker and Riker (33), were made and incubated at 20° C., but very few spores formed. Cultures on steamed potato plugs gave only slightly better sporulation.

Many conidia were formed, however, in about 10 weeks on potato-dextrose agar cultures of various Wisconsin isolates grown at low temperatures (4° to 8° C.). Equally good results were obtained in about 8 weeks when cultures were started at 16° to 20° and transferred to 4° C. after 10 to 15 days. This treatment made possible the production of large amounts of conidial inoculum at any season, but required the maintenance of many cultures.

Conidia of *Sclerotinia laxa* were formed also on inoculated apples and pears. Wisconsin isolate 1, when inoculated into overripe Bosc pears and incubated in a moist chamber at 20° C., produced well-developed sporodochia within 60 hours. Under similar conditions small sporodochia were formed on Emperor grapes in 36 hours. On apples sporulation was slow and variable. Many sporodochia were produced on 3 of 5 inoculated Jonathan apples in 5 days at 20° C., but very few spores appeared on 5 Wealthy, 5 Red Delicious, and 10 McIntosh apples similarly treated. Good sporulation occurred on fresh sour cherries, but not on canned or frozen sour cherries, frozen peaches, or on dried prunes.

The best yield of conidia was obtained by growing the fungus on Emperor grapes. For best results the grapes were used raw or heated to 60° C. for 10 minutes. Relatively few conidia formed on autoclaved grapes. With the exception of a few isolates from California, abundant sporulation developed in 3 or 4 days in cultures on slightly heated grapes.

Microconidia, almost spherical and about 2.5 μ in diameter, were produced in tremendous numbers by most isolates of *Sclerotinia laxa* on various substrata under a variety of environmental conditions. The microconidia are formed in chains on tiny sterigmata borne by club-shaped structures. They are imbedded in a matrix and appear on the surface of a culture as slimy masses of variable color, usually cream, yellow, dark green, or black. The sexual function of microconidia of *S. Gladioli* (Massey) Drayton has been discussed by Drayton (12), but the sexual nature of microconidia of *S. laxa* has not been demonstrated.

Apothecia of *Sclerotinia laxa* have not been produced in culture on artificial media. Their occasional occurrence on mummified fruits has been reported from Europe (1) and the British Isles (40), but not from North America. Stalked structures that suggest apothecial fundaments, $\frac{1}{4}$ to $\frac{1}{2}$ inch high, formed in some cultures of isolate 62 (Washington) and of isolate 21 (Wisconsin) but failed to mature into apothecia.

Temperature relationships. The effect of temperature on the radial growth rates of *Sclerotinia laxa* isolates 2, 21, and 23 was studied briefly, and comparative tests were made with *S. fructicola* isolates 4 and 12. Each culture was grown on 20 cc. of 2 per cent potato-dextrose agar in a Petri dish. The cultures were started by placing a standardized seeded disc, 3 mm. in diameter and 2 mm. thick, of 2.5 per cent dextrose agar at the center of each Petri dish. Triplicate cultures of each isolate were incubated at 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 37° C. Measurements of radial growth were made after 2, 4, 7, 14, and 30 days. Radial growth of the 3 *S. laxa* isolates and isolate 12 of *S. fructicola* in 4 days was greatest at 28°, whereas *S. fructicola* isolate 4 grew most rapidly at 24° C. In 7 days *S. fructicola* grew more than *S. laxa* at 12°, 16°, 20°, and 24°, but less at 4° C. At 8° C. *S. fructicola* isolate 4 grew faster and isolate 12 slower during the first week than any of the 3 *S. laxa* isolates, but in 2 weeks both *S. fructicola* isolates were far ahead of *S. laxa*. At 28° C. differences in growth rates between isolates of the same species were greater than between certain isolates of different species. At 32° C. *S. fructicola* grew more rapidly than *S. laxa*. No growth of either occurred at 37° C.

Conidial size. Conidia of numerous isolates of *Sclerotinia laxa* and *S. fructicola* had the same general characteristics when examined microscopically. They formed in branched chains without true disjunctors, but, when mature, they broke apart easily at the middle of wedge-like thickenings between the spores. As the spores separated from each other, they became roughly lemon-shaped, although there was considerable variation in relative dimensions, so that some were nearly spherical.

Measurements of conidia from various sources were made, and the average dimensions are recorded in table 1. Each solitary spore in fields chosen at random was measured in order to obtain valid average dimensions. Figures obtained compare favorably with those given by other workers (7, 13, 25, 27, 31, 34, 42). Considerable variation in spore size was apparent between samples produced at different temperatures or on different substrata. Spores produced on dead, overwintered plant parts were usually smaller than those formed on fresh fruit or on artificial media. Those formed at low temperatures tended to be smaller than those produced at 20° to 25° C. Similar phenomena have been noted by Hewitt and Leach (16), Killian (24), Ritzema Bos (34), and Wormald (42). It is apparent that conidial

TABLE 1.—*Measurements of conidia of Sclerotinia laxa and S. fructicola*

Species, isolate, and source	Substratum	Temperature	Average dimensions[a]
		°C.	*Microns*
S. laxa			
2 *Prunus cerasus* twig, Wis.	Steamed potato plug	22	13.9 × 18.5
2 Do	Emperor grape	22	10.8 × 16.1
21 Do	Potato-dextrose agar	20	10.9 × 14.8
23 Do	Sour cherry blossom	.....	12.8 × 17.5
23 Do	Potato-dextrose agar	20	10.7 × 14.9
23 Do	Do	4	9.3 × 12.0
100 Do	Sour cherry twig	.....	9.1 × 13.2
101 *Prunus cerasus* green fruit, Wis.	Green sour cherry fruit	.....	11.7 × 14.4
87 *Prunus pennsylvanica* twig, Wis.	Emperor grape	22	9.3 × 13.7
50 *Prunus domestica* mummy, Ore.	Potato-dextrose agar	22	8.8 × 14.1
102 Do	Italian prune mummy	.....	7.3 × 10.6
S. fructicola			
4 *Prunus domestica*, Wis.	Potato-dextrose agar	22	9.2 × 12.8

[a] Figures based on measurements of 100 spores per sample. See text.

measurements alone provide insufficient basis for differentiation between *Sclerotinia laxa* and *S. fructicola.*

Spore germination. Branched and crooked growth of the germ tubes of *Sclerotinia laxa* soon after conidial germination has been mentioned by Ezekiel (13), Roberts and Dunegan (35), and Wormald (44) as a characteristic of this species useful in distinguishing it from *S. fructicola.* Ezekiel germinated spores of each species in hanging drops of potato-dextrose decoction at 25° C., and after 18 hours found the germ tubes of *S. laxa* to be branched and crooked, whereas those of *S. fructicola* were typically unbranched and straight.

Experiments made in this laboratory reveal that characteristic germ tube differences between the two species may not occur under certain conditions. Conidia of *Sclerotinia laxa* isolate 23 and *S. fructicola* isolate 12 were germinated over deep-well slides in hanging drops of distilled water and in 0.1 and 0.01 per cent dextrose solutions. Very little branching occurred in any

drop, and the only difference noted was the slightly more tortuous nature of some *S. laxa* germ tubes. Characteristic differences in branching were noted, however, when conidia of the two species were made into spore suspensions over grape cultures upon which they had been grown. When drops of these spore suspensions, which contained considerable nutrient material from the grapes, were incubated at various temperatures from 12° to 32° C., the *S. laxa* conidia produced crooked, branched germ tubes quickly, while the germ tubes of *S. fructicola* usually remained straight for 24 hours or more. It is evident from these tests that growth-habit differences between the germ tubes of the two species are readily apparent only under certain nutritional conditions favorable to their expression.

Conidia of *Sclerotinia fructicola* germinated more quickly in distilled water or weak sugar solutions than conidia of *S. laxa*. *S. laxa* conidia, regardless of age, required at least 4 hours for germination in distilled water at 24° C., whereas conidia of *S. fructicola* only 2 days old germinated under the same conditions in 75 minutes. The addition of 0.1 per cent dextrose to distilled water reduced the minimum time for conidial germination of *S. laxa* to about 2 hours at 24° C.

The optimum temperature for *Sclerotinia fructicola* conidial germination was 24°, but for *S. laxa* 20° C. appeared slightly more favorable. Spores of both species germinated readily from 16° to 32° C. Some germination occurred at 4° and at 34° but not at 37° C.

DISEASE DEVELOPMENT STUDIES

Methods and materials. All blossom inoculations were made on trees 2- to 4-years-old set in 8-inch pots at least 14 months before inoculation and forced into bloom in the greenhouse about February 1. Blossoms were inoculated, except as otherwise stated, by means of a conidial suspension sprayed from an atomizer attached to a compressed-air line. All spore suspensions used in large-scale inoculations were mixed from 2 or 3 isolates. In the hope of obtaining an inoculum more representative of the species, mass-isolated rather than single-spore cultures were used for inoculation work.

Immediately after inoculation, trees were placed in a saturated atmosphere in large moist chambers. Two types of chamber were used. One type, which provided condensation water adequate for infection and disease development has been described by Keitt and Jones (20). The second type, of temporary construction, made use of mist to maintain high atmospheric moisture. A fine, relatively uniform mist was produced by means of atomizers connected to about 20 pounds air pressure and water reservoirs. To reduce temperature fluctuations, 1 or 2 thermostatically controlled, 250-watt, gooseneck heaters were installed in each mist-type moist chamber, except the one at 11° C. where no heater was necessary. Temperature variations were about plus or minus one degree centigrade except in the 11° house, which, though usually between 10° and 12°, occasionally reached 13° C.

Inoculated trees were kept in a moist chamber at least 3 days to permit the fungus to become well established in the blossoms. After this, in 1944, they were allowed to dry about 8 hours (4:00 p.m. to midnight) each day. It was thought, however, that a proportionately greater amount of time in a wet environment might favor more severe attacks of brown rot. Therefore, in 1945 the spray was turned off only about 8 hours (9:00 a.m. to 5:00 p.m.) every second day, and once each week the dry period was lengthened to 24 hours to permit thorough drying of the trees and wilting of diseased branches. The same treatment was accorded chambers at all temperatures.

TABLE 2.—*Relation of temperature to infection of sour cherry blossoms by Sclerotinia laxa*

Year and variety	Incubation temperature	Blossoms inoculated[a]	Blossoms infected[b]
	°C.	*Number*	*Per cent*
1944	Initial[c]		
Montmorency	10	272	14
	20	152	72
	30	218	94
	Secondary[d]		
Montmorency	11	126	40
	16	101	44
	20	163	52
	24	155	68
	28	97	69
	Continuous[e]		
1945	11	187	81
Montmorency	16	132	92
	20	142	97
	24	186	98
Early Richmond	11	52	94
	16	167	96
	20	219	94
	24	128	98

[a] Includes all open blossoms, with or without petals.
[b] Data taken 5 days after inoculation in 1944, and at 5, 6, 7, and 13 days at 11°, 16°, 20°, and 24° C., respectively, in 1945.
[c] Temperature during first 48 hours after inoculation.
[d] Temperature maintained following initial 48-hour incubation (see text).
[e] Temperature maintained from inoculation.

Preliminary Experiment in 1943. In March, 1943, a preliminary greenhouse experiment demonstrated the pathogenicity of Wisconsin isolate 1 of *Sclerotinia laxa* on blossoms of Montmorency and Bing cherries and on blossoms of Hale Haven peach.

Experiments in 1944. The main experiment in 1944 was confined to Montmorency blossoms because no Early Richmond blossoms were available. Blossoms were inoculated with mixed conidial suspensions of 3 *Sclerotinia laxa* isolates known to be virulent and given a 48-hour incubation period in a chamber which provided condensation water adequate for infection. Incubation temperatures used during this period were 10°, 20°, and 30° C.

After 48 hours the trees were removed to the mist-type moist chambers. Blossoms from each of the 3 groups (10°, 20°, 30° C.) were distributed as evenly as possible among the 5 chambers (at 11°, 16°, 20°, 24°, and 28° C.). Blossom infection data were taken 5 days after inoculation, but before the appearance of secondary infection.

The results, which are shown in table 2, are compiled separately according to the initial temperature (in the inoculation chamber) and the secondary or later incubation temperature. It is recognized, however, that in this experiment the effects of the initial and the later incubation temperatures cannot be sharply differentiated.

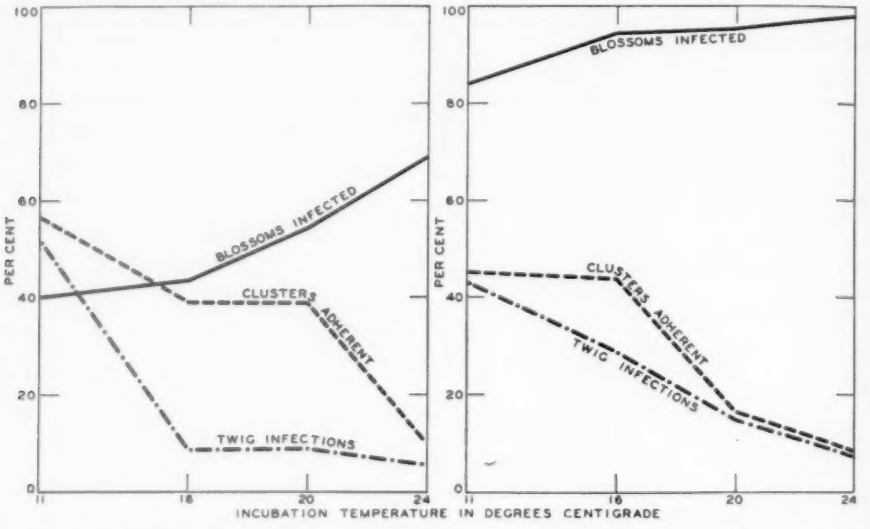

FIG. 5. Results of inoculations of young sour cherry trees with *Sclerotinia laxa* conidia, in the greenhouse, showing (a) percentages of primary blossom infection, (b) blossom clusters adherent to the twigs 6 weeks after inoculation, and (c) blossom clusters from which twigs became infected within 8 weeks following inoculation. Data were taken in 1944 (left) from Montmorency only, and in 1945 (right) from Montmorency and Early Richmond varieties.

Thirty degrees centigrade appeared to be the incubation temperature (first 48 hours) most favorable to blossom infection and 10° least favorable. Of the 5 temperatures used after the initial incubation period, 11° seemed to be least favorable and 24° and 28° C. most favorable.

Because only a relatively small number of blossoms was moved from each of the 3 inoculation groups to each of the 5 temporary moist chambers, no detailed table of results from these 15 subdivisions is presented. However, it was found that only 2 of 62 blossoms inoculated at 10° and kept at 11° C. were diseased in 5 days, whereas 97 of 99 blossoms inoculated at 30° and removed after 48 hours to 24° or 28° C. were infected.

It was observed that many infected blossom clusters were abscised prior to twig infection. Therefore, records were taken in all chambers to determine (a) the number of blossom clusters adherent to the trees 6 weeks

after inoculation and (b) the number of clusters from which the fungus had entered the wood or bark of the supporting twig within 8 weeks after inoculation. The results of this study are shown graphically in figure 5.

The critical period for blossom infection, on the basis of the studies in 1944, begins with the opening of the blossoms and continues until the floral cups turn yellow and wither (Table 3). At ordinary temperatures blossoms remain susceptible 1 to 2 weeks.

Although few yellowed blossoms were inoculated, and though such blooms were very susceptible to attack through withered and damaged stamens, it was observed that the infection spread so slowly in the floral cups and pedicels that it usually did not reach the base of the pedicels before the blossoms dropped.

Experiments in 1945. Both Early Richmond and Montmorency blossoms were available for inoculation in 1945, and improved facilities per-

TABLE 3.—*Incidence of primary infection by Sclerotinia laxa of sour cherry blossoms inoculated at different stages of development*

Year and variety	Floral development[a]	Blossoms inoculated	Blossoms infected[b]
		Number	*Per cent*
1944	Petals unopened	243	2
Montmorency	Petals opened	389	53
	Petals fallen	253	57
1945	Green-tipped bud	119	10
Early Richmond	White-tipped bud	355	30
	Petals opened	994	95
	Petals fallen	283	91

[a] At hour of inoculation.
[b] Data taken according to statement in text.

mitted the distribution of trees of each variety among the various incubation temperatures immediately after inoculation. The concentration of spores in the inoculum was doubled over that used in 1944. Spore suspensions were prepared from conidia produced on grape cultures.

Records were taken on each variety to determine (a) the number of blossoms showing primary infection at different temperatures, (b) the number of buds or blossoms infected after inoculation at different stages of development, (c) the part or parts of blossoms where infection occurred, (d) the number of blossom clusters adherent after 6 weeks, and (e) the number of blossom clusters from which the fungus entered the wood within 8 weeks (Fig. 5).

The numbers of blossoms infected at different temperatures are shown in table 2. In order to avoid including secondary infections, the counts were made 5, 6, 7, and 13 days after inoculation at 24°, 20°, 16°, and 11° C., respectively. Except at 11° C., there was little apparent difference in varietal susceptibility. High percentages of infection were obtained at all temperatures, particularly on Early Richmond, but with Montmorency

blossoms there appeared to be a slight trend to more infection at higher temperatures. The increase in amount of infection obtained in 1945, over that in 1944, is attributed chiefly to the increased spore load of the inoculum.

The critical period for infection, as in the 1944 experiment, began with the opening of the blossoms. Although some buds inoculated while still in the green-tip stage were infected, it was observed that no infection occurred until after the petals were exposed. Infection of white-tipped buds usually followed the opening of the flowers, but sometimes took place earlier through the exposed outer surfaces of the petals. The data on infection are based on the stages of blossom development at inoculation rather than at infection.

Results from both varieties have been combined in table 3. No important varietal differences were apparent in this experiment, although a slightly higher percentage of infection occurred with Early Richmond than with Montmorency. The site of blossom infection was almost invariably on an anther, stigma, or petal. In this connection anthers and stigmas appeared to be of greater importance than petals, which though often infected, usually dropped before the pathogen entered the rim of the floral cup. Primary infection of other flower parts was rarely seen. The frequency of infection through anthers and stigmas of 813 perfect flowers is shown in table 4.

Microscopic examinations of infected anthers consistently revealed germinated pollen and fungus hyphae, and sometimes fungus hyphae passing from the anthers into the pointed tips of the filaments. Almost pure cultures of *Sclerotinia laxa* were isolated repeatedly from diseased anthers.

A special experiment was conducted to determine by direct inoculation of selected floral parts those through which the fungus is most likely to enter the flower.

Inoculations were made by application either of dry conidia or droplets of conidial suspension to specific floral parts. Certain unwanted blossom structures were removed before inoculation. Stamens of blossoms receiving style inoculations were cut off, and the number of stamens was reduced to five on blossoms receiving filament or anther inoculations. Anthers were removed from all inoculated filaments. In this experiment all trees were incubated at 20° to 24° C. in a moist chamber which provided sufficient condensation moisture for infection, but not enough to permit the formation of large drops which might have displaced the inoculum.

Stigma inoculations gave the highest percentage of infections; anthers the next highest (Table 5). It was noted that unruptured anthers rarely were invaded, while uninjured petals were found to be somewhat susceptible. Styles were rarely attacked directly, and no direct infection of filaments was observed. However, 24 styles, each inoculated about 3 millimeters below the stigma, became infected later by way of the stigmas. Only 1 sepal, which had been injured, was infected.

TABLE 4.—*Incidence of primary infection through anthers and stigmas of Early Richmond and Montmorency sour cherry blossoms sprayed with a conidial suspension of Sclerotinia laxa, 1945*

Variety	Blossoms inoculated	Blossoms infected[a]			
		Via anthers only	Via stigma only	Via anthers and stigma	Total
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Early Richmond	353	34.6	0.0	57.5	92.1
Montmorency	460	41.3	0.7	48.9	90.9
Total	813	38.4	0.4	52.6	91.4

[a] Data taken soon after appearance of primary lesions.

Considerable leaf infection developed at 16°, 20°, and 24° C. The greatest amount occurred at 20°, but very little occurred at 11° C. It is possible that inoculum may have been driven into the leaf tissues by the force of the spray from the atomizer, for no leaf infection developed on 50 sound leaves inoculated with drops of spore suspension carried on a wire loop. Most leaf infection seen on orchard trees followed direct contact of the leaf with an infected blossom or blossom part, but leaf lesions similar to those developed in the greenhouse (Fig. 2, D) have been described and illustrated by Maier and Mittman-Maier (26) on sweet cherry.

The rate of disease development at 5°, 11°, 16°, 20°, 24°, and 28° C. was studied. These standard temperatures were maintained within 1° C., except in the 11° C. chamber where occasional variations of 2° C. occurred. The approximate incubation period required for the development of certain symptoms at each of the above temperatures was determined (Table 6). The disease progressed most rapidly in the blossoms at 24° and was but little slower at 20° C. At 5° C. no infection was visible for 6 days, and 9 more days were required for production of lesions on the rim of the floral cup. There was practically no difference between Early Richmond and Montmorency varieties in the rate of disease development on the blossoms.

TABLE 5.—*Incidence of infection by Sclerotinia laxa of various specially inoculated parts of Early Richmond and Montmorency sour cherry blossoms, 1945*

Floral part inoculated	Inoculum	Units inoculated	Units infected[a]
		Number	*Per cent*
Stigma	Dry conidia	13	92
Stigma	Conidial suspension	6	100
Anther	Dry conidia	40	35
Style	Conidial suspension	50	10
Filament	do	78	0
Petal (outer surface)	do	15	0
Petal (inner surface)	do	15	20
Sepal	do	50	2[b]

[a] Final readings made 8 days after inoculation.

[b] This sepal showed evidence of mechanical injury.

Sclerotinia laxa apparently required a considerably longer incubation than *S. fructicola*. Weaver (37) reported infection of peach blossoms by *S. fructicola* only 5 hours after inoculation at 24°, and 18 hours after inoculation at 10° C.

CONTROL

Preliminary experiment in 1942. A preliminary experiment on the control of spur blight was made in an orchard which had been severely damaged by *Sclerotinia laxa* in 1941. Materials used were (a) Elgetol (sodium dinitro-ortho-cresolate), 0.5 per cent, (b) Fermate (ferric dimethyl dithiocarbamate), ¾–50, (c) copper-lime-monocalcium arsenite and cold-pressed menhaden fish oil, 3–2–2 (1½ pints fish oil) –50, and (d) Bordeaux mixture, 3–4–50. The selection of the formula for the copper-lime-arsenite mixture

TABLE 6.—*Approximate minimum incubation periods for appearance at stated temperatures of certain symptoms on Early Richmond and Montmorency sour cherry blossoms inoculated with conidia of Sclerotinia laxa*

Variety	Incubation temperature	Minimum period for expression of stated symptoms			
		Tips of styles or filaments browned	Lesions on calyx tube	Tip of pedicel browned	Entire pedicel[a] browned
	°C.	*Hours*	*Hours*	*Hours*	*Hours*
Early Richmond	5	144	360	432	504
	11	48	132	152	186
	16	36	62	90	160
	20	15	38	58	87
	24	14	35	52	80
	28	20	44	70	108
Montmorency	5	144	360	432	504
	11	48	132	152	186
	16	36	62	90	175
	20	20	42	63	100
	24	16	35	52	80
	28	20	44	70	108

[a] Pedicel length approximately 25 mm.

was based on results obtained by Keitt and Palmiter (22). Spraying was unavoidably delayed until May 16, just as the trees were breaking into bloom.

The copper-lime-monocalcium arsenite mixture stopped sporulation almost completely and gave visibly better results than Elgetol. Bordeaux mixture and Fermate appeared to reduce the number of blighted spurs, but neither halted the formation of sporodochia.

The application of Elgetol or copper-lime-arsenite to the trees during bloom resulted, as expected, in considerable injury to the blossoms and young leaves, but the twigs were not severely damaged. The suppression of sporodochial production and the absence of serious injury to the trees by copper-lime-monocalcium arsenite indicated its probable value as an eradicant dormant spray for the control of *Sclerotinia laxa*.

Experiments in 1943. Control work was continued in 1943 on Early Richmond trees about 15 years old in an experimental area containing 5 blocks of 24 trees each and one of 6 trees. Tests were confined to 2 eradicant materials, applied just before budbreak, and Bordeaux mixture, 3–4–50, applied early in bloom.

An eradicant spray of copper-lime-monocalcium arsenite and fish oil, 3–2–2 (1½ pints fish oil) –50 was applied to blocks II and III. In the preparation of this material the copper sulphate and then the lime were put in the tank to form Bordeaux mixture. Next the monocalcium arsenite and finally the fish oil were added. The 6 trees in block I received monocalcium arsenite, 2–50, without Bordeaux mixture or fish oil. Blocks IV and V received no dormant spray. The eradicant dormant sprays were applied May 6, when a few buds were beginning to swell. Thorough applications, with considerable run-off, were made in an effort to bring the spray materials into direct contact with all diseased parts.

A protectant spray of Bordeaux mixture, 3–4–50, was applied June 1 to blocks III and IV, when about three-fourths of the blossoms were open. All experimental spraying was done from the ground. Block V was used as an unsprayed check.

Data were taken on sporodochial suppression and on incidence of spur blight from 6 representative trees in each of the 5 blocks. On June 8, at the end of the critical period for infection, 10 overwintered, diseased spurs were collected at random from each of these trees. Subsequently, counts of sporodochia were made on 30 spurs taken at random from each sample. These counts included sporodochia on or within adherent flowers, fruits, leaves, bracts, and bud scales.

The blossom spray alone had little or no effect on the suppression of sporodochia, but the eradicant dormant sprays proved very effective. Monocalcium arsenite, 2–50, on the basis of these counts, reduced the production of sporodochia 83 per cent; but the copper-lime-monocalcium arsenite and fish-oil mixture was significantly more effective, with 94 per cent sporodochial suppression (Table 7).

Counts of blighted spurs were obtained July 2 by examination of a total of 300 spurs, not over 3 inches in length, on each of 6 trees per block (Table 8). Spurs counted were on branches taken at random around the perimeter of each tree.

The incidence of spur blight was greatly reduced in all sprayed blocks. The use of copper-lime-monocalcium arsenite and fish-oil preparation followed by the bloom spray (block III) gave the most striking results, 99 per cent reduction in incidence of spur blight. However, an analysis of variance of figures for spurs blighted in blocks II to V, inclusive, showed no significant differences between results for blocks II and III. Control either in block II or block III was significantly better than in block IV at odds of 19 to 1. Block I was not comparable, so could not be included

TABLE 7.—*Effectiveness of eradicant dormant sprays in limiting the sporodochial production of Sclerotinia laxa on overwintered dead spurs of Early Richmond sour cherry trees, 1943 and 1944*

Block and treatment[a]	Spurs examined[b]		Average sporodochia per spur		Indicated reduction in sporodochia by spray	
	1943	1944	1943[c]	1944[d]	1943[c]	1944[d]
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
I Monocalcium arsenite	30	.....	7.5	.....	83	.....
II Copper-lime-monocalcium arsenite + fish oil	30	60	2.8	4.1	94	85
VI Copper-lime-monocalcium arsenite + dormant spray oil	.....	60	.....	0.7	.....	97
V None	30	60	43.1	26.2	.....	.....

[a] Dates of application: May 6, 1943; April 30, 1944. See text for dosages.
[b] Dates of spur collection: June 8, 1943; June 22, 1944.
[c] Data based on counts of total sporodochia on 30 spurs from each treatment. See text.
[d] Data based on counts of total sporodochia on 60 spurs (10 from each of 6 trees) per treatment.

in the analysis of variance. Counts on 2 trees in block I showed approximately 7 per cent of the spurs blighted.

Slight injury developed on some trees receiving the dormant spray alone. Twig and branch injury was confined to weak or wounded trees.

Experiments in 1944. These experiments were made on Early Richmond trees about 15 years old in an orchard approximately ½ mile from the 1943 plots. The experimental area was divided into 5 blocks of 20 trees each, plus a sixth block containing only 12 trees.

Two eradicant spray preparations were tested in 1944. Copper-lime-monocalcium arsenite and fish oil 3–2–2 (1½ pint fish oil) –50, was applied to all trees in blocks II and III. Copper-lime-monocalcium arsenite and Stanolind dormant spray oil, 3–2–2 (1½ pints fish oil) –50, was used on the 12 trees in block VI. This mixture was included in the experiment in the hope

TABLE 8.—*Effectiveness of early sprays in the control of brown-rot spur blight on Early Richmond sour cherry trees, 1943*

Block and treatment[a]	Spurs examined[b]	Spurs blighted		Indicated reduction by spray
	Number	*Number*	*Per cent*	*Per cent*
II *Dormant*, copper-lime-monocalcium arsenite + fish oil	1800	23	1.3	97.5
III *Dormant*, copper-lime-monocalcium arsenite + fish oil; *Bloom*, Bordeaux mixture	1800	9	0.5	99.1
IV *Bloom*, Bordeaux mixture	1800	170	9.4	82.1
V None	1800	948	52.7	.....

[a] Dates of application: Dormant, May 6; Bloom, June 1. See text for dosages.
[b] Data taken on 300 spurs per tree. See text.

of finding a suitable oil to replace the fish oil component in the dormant spray. Monocalcium arsenite, 2–50 (block I), because of its inferior record of sporodochial suppression in 1943 was not included in the 1944 program. The dormant sprays were applied April 30 when the buds were just beginning to swell.

Bordeaux mixture, 3–4–50, was the only protectant bloom spray used. It was applied to the trees in blocks III and IV on May 28, when only about one-fourth of the blossoms were open, and was used as a late-bloom spray in block VII on June 2, after about two-thirds of the petals had fallen.

Data were taken on sporodochial suppression, incidence of blossom blight, and incidence of spur blight. Counts of sporodochia were made only in blocks II, V, and VI. Ten overwintered, blighted spurs were col-

TABLE 9.—*Effectiveness of early sprays in the control of brown-rot blossom blight on Early Richmond sour cherry trees, 1944*

Block and treatment[a]	Blossoms examined[b]	Blossoms infected	
	Number	*Number*	*Per cent*
II *Dormant*, copper-lime-monocalcium arsenite + fish oil	1800	17	0.9
VI *Dormant*, copper-lime-monocalcium arsenite + dormant spray oil	1800	8	0.4
III *Dormant*, copper-lime-monocalcium arsenite + fish oil; *Early bloom*, Bordeaux mixture	1800	20	1.1
IV *Early bloom*, Bordeaux mixture	1800	27	1.5
VII *Late bloom*, Bordeaux mixture	1800	83	4.6
V None	1800	79	4.4

[a] Dates of applications: Dormant, April 30; Early bloom, May 28; Late bloom, June 2. See text for dosages.
[b] An equal number of blossoms was examined from 6 trees per block.
[c] Minimum significant difference at odds of 19 to 1 = 28.7.

lected at random from each of 6 trees per block and examined to determine the numbers of sporodochia present (Table 7).

Either dormant spray preparation provided good suppression of sporodochia, although a few sporodochia were present on some spurs when the sprays were applied; but the mixture containing dormant spray oil was significantly more effective at odds of 19 to 1 than that made with fish oil. Very little injury was apparent from either treatment.

Blossom blight data were taken in every block. On June 4, four days after the beginning of the first infection period, 300 blossoms from clusters selected at random were examined on each of 6 trees per block. Despite the brief infection period substantially fewer blighted blossoms were found in all sprayed blocks, except block VII, which was sprayed after the infection period, than in unsprayed block V (Table 9). Differences among blocks II, III, IV, and VI were slight, as might be expected in mild attacks.

There was little blossom blight or spur blight in 1944 because dry weather prevailed during the critical period. Only 2.8 per cent blighted spurs were

found in the unsprayed block, and the most severely diseased unsprayed tree had only 9 per cent blighted blossoms. In order to obtain any indication of the measure of control by different treatments, it was necessary to determine the total number of blighted twigs (spurs and terminals) on each of the trees selected from each block. On the basis of these figures the dormant spray with a supplementary bloom spray, as in 1943, gave the best control (92 per cent). Bordeaux mixture during early bloom apparently reduced spur blight about 60 per cent, whereas the dormant spray alone reduced it only 50 per cent. No spur blight was found in block VI where the copper-lime-monocalcium arsenite plus dormant spray oil was used. The late bloom spray in block VII gave no reduction of blossom or spur blight.

DISCUSSION

The existence of biotypes intermediate between typical isolates of *Sclerotinia laxa* and *S. fructicola* is apparent (Fig. 4). Ezekiel (13) illustrates several cultural types of each species. Hewitt and Leach (16) show a culture of *S. fructicola* which, though it has the usual concentric rings and considerable sporulation, has also an irregular, somewhat lobed margin like *S. laxa*. On the other hand, many lines of *S. laxa* isolated by us from specimens obtained from California and Oregon formed many aerial hyphae but sporulated less readily than *S. fructicola*.

Wisconsin isolate 4 obtained from a plum mummy near Madison, where no *Sclerotinia laxa* has been found, appeared in culture as a typical colony of *S. fructicola* at 12° to 32°, but at 4° and 8° C. it formed lobes and zones closely resembling *S. laxa* (Fig. 3). However, isolates from the margins of such lobes reverted to the typical *S. fructicola* growth habit when incubated at higher temperatures. Thus, at least with this isolate, the growth habit of *S. fructicola* appeared almost identical with that of *S. laxa* at 4° or 8° C. No instance of a similar change of growth habit was observed in *S. laxa*.

The stability in culture of various isolates of *Sclerotinia laxa* and *S. fructicola* suggests the possible genetic nature of certain variants. Anastomoses between different lines may possibly result in new and intermediate types. Intraspecific anastomoses are common, and Ezekiel (13) observed an interspecific anastomosis between *S. laxa* and *S. fructicola*. Moreover, natural mating of *S. laxa* and *S. fructicola* in areas where both species occur is a possibility not seriously discounted by the fact that no apothecia of *S. laxa* have been reported in North America. Experimental interspecific and intraspecific matings of these fungi may eventually do much to clarify this matter.

The results of the present work indicate that moderate to high temperatures (16° to 30°) are more favorable to blossom blight than temperatures of 11° C. or lower, and that infection and disease development on blossoms are most rapid at about 24° C. However, it should be noted, that although

the progress of the disease was retarded by low temperatures, it was possible to obtain a high percentage of infected blossoms at 11° C., particularly with a concentrated spore suspension such as that used in 1945.

Temperatures while the fungus is invading the blossoms probably are no more important than those prevailing during subsequent dry periods. The occurrence of warm weather after the blossoms have been destroyed but before the fungus has entered the wood of the supporting twigs favors rapid drying of the diseased parts and the maturation of abscission layers at the bases of the pedicels and, thereby, the dropping of the diseased flowers. Even when the environment is almost continuously moist and the temperature relatively constant, as in the incubation chambers, the results indicate that blossom clusters are, eventually, more subject to abscission at high or moderate than at low temperatures (Fig. 5).

The effect of different temperatures on the spur-blight phase of brown rot, because of the small number of spurs blighted in the greenhouse, was less striking than for blossom blight. However, at 11° C. there was less abscission of diseased blossoms and, consequently, more spur blight than at higher temperatures.

A preliminary experiment with *Sclerotinia laxa* blossom blight under conditions of controlled humidity gave results agreeing closely with those obtained by Weaver (37) for *S. fructicola* blossom blight on peach and cherry. Weaver found that at relative humidities above 90 per cent the diseased areas remained fairly moist, whereas at 80 per cent the lesions dried out and were delimited. It is apparent that while the blossom-blight phase is greatly favored by constant conditions of abundant moisture and moderate temperature, the spur-blight phase of European brown rot probably develops more readily under a complex of fluctuating moisture and temperature conditions.

The respective lengths of moist and dry periods necessary for extensive development of spur blight were not investigated, but it was observed that under conditions prevailing in the incubation chambers the fungus usually grew no farther than the short axis within the bud scales. It was observed that very little additional moist weather was necessary for the development of spur blight after the fungus had rotted the pedicels.

Many statements concerning the court of blossom infection for European brown rot of stone fruits are found in the literature. Woronin (45) considered the stigma to be the only normal infection court. This opinion was accepted by most pathologists until Wormald (39) in 1919 obtained infection of plum flowers by placing spores at the bottom of the floral cups, on the "discs" and on the stigmas. Chabrolin (10) in 1924 reported that under natural conditions infection of apricot blossoms arises through the calyx, particularly through the tube, and through the filaments of the stamens as well as through the stigma. He believed that, because of their much smaller area, the stigmas were less frequently infected than the

floral envelope, but he lacked sufficient evidence to prove it. He found hyphae present in the anthers between the pollen grains, but apparently he did not consider the anthers to be infection courts. Maier (25) in 1942 recognized that infection may occur through either the stigma or the receptacle. Weaver (37) found that *Sclerotinia fructicola* was capable of infecting all floral organs of peaches; but no reference to anthers as important infection courts for *S. laxa* has been found. However, according to the experiments reported herein, blossom infection originates in anthers more often than in any other floral part; but the progress of the fungus as it travels downward through one or several filaments, usually somewhat obscured by about 30 other stamens, is not easily observed. The true nature of most "floral-cup" or "receptacle" infections was revealed only by a series of careful observations beginning not more than 14 hours after inoculation. Under close scrutiny nearly all such infections have been found to originate in the anthers. Although experiments indicated that a lower percentage of inoculated anthers than stigmas was attacked (Table 5), the greater number of anthers (25 to 35) per flower rendered blossom infection through one or more anthers more probable than stigma infection. Even though 10 per cent of 50 styles experimentally inoculated became infected, these infections were slow in appearing, and had the anthers or stigmas of these flowers been inoculated at the same time as the styles, the styles would have rotted from above or below before infection occurred through their own surfaces. Most blossom infections, by natural or by artificial inoculations, originated in an anther or a stigma, where the host tissues were not resistant to attack and a plentiful food supply was available to the developing hyphae.

Early Richmond trees were used in the spraying experiments because they had been observed in Wisconsin, and reported by Huber and Baur (19) in Washington, to be much more severely attacked than Montmorency by *Sclerotinia laxa*. Treatments were applied to blocks of trees rather than to single-tree plots to reduce the influence of air-borne conidia from near-by trees receiving different treatments.

The selection of spray materials and schedules tested in 1943 was based upon a study of the usual control practices for *Sclerotinia laxa* in Europe and North America and upon consideration of the promising results of experiments with eradicant fungicides in Wisconsin (22) and California (38). Recommended control measures for *S. laxa* in England (11, 29) include (a) the destruction of the diseased plant parts, (b) a dormant spray of 10 per cent tar oil before the buds begin to swell, and (c) a spray of Bordeaux mixture (about 3–4½–50) just before the blossoms open. Anres and Joessel (2) advised sprays of Bordeaux mixture, 2 per cent, on apricots in France when the buds are opening and at the beginning of blossoming. In Germany, Bucksteeg (8) suggested 4 sprays of unspecified materials to be applied (a) when the buds begin to swell, (b) just before

the blossoms open, (c) just after petal fall, and (d) 2 or 3 weeks after petal fall. Rudolph (36) in 1925 reported that in California insecticide oil sprays applied to apricots during the winter frequently showed fungicidal properties. For blossom blight control he advised a dormant spray of crude-oil emulsion, 30–200, a dormant spray of Bordeaux mixture, 8–8–50, and 1 or 2 applications of Bordeaux mixture of various strengths during bloom.

Keitt and Palmiter (22) in 1937 reported the results of tests made over a 13-year period with various eradicant fungicides. They found that suitable copper-lime-arsenite mixtures were highly effective against fungal fruiting structures at the surface or near a permeable surface. Wilson (38) reported in 1942 the results of experiments with arsenite sprays to control *Sclerotinia laxa* on almonds, apricots, plums, and prunes in California. He found monocalcium arsenite, 2 or 4 pounds per 100 gallons, to be an effective eradicant spray.

Less emphasis is placed on the results of the control experiments in 1944 than in 1943 because of the sparse development of lesions in 1944. In 1943 there was an almost continuous 5-day infection period early in bloom, with considerable wind and rain. Following this the weather was almost ideal for spur blight development. In 1944, however, although abundant inoculum was present because early rains had favored sporulation, the blossom period was so dry that little infection occurred. There was only one light shower (about 0.2 inch) during bloom.

The proper time for application of the dormant spray in Door County is usually limited by weather and orchard conditions to a period of 1 to 2 weeks before budbreak. Moreover, the growth cycle of the fungus in that area is almost perfectly adapted to the use of an eradicant spray during that period. Sporodochia of *Sclerotinia laxa* apparently are formed in Door County only in the spring. In California and Oregon they may be formed soon after the fall rains begin, but this phenomenon has not been observed in Wisconsin, where sporodochial formation begins after the spring rains start (in 1942–1944 about 7 to 10 days before the buds began to swell) and continues for a month or more.

The advisability of using two special sprays, rather than one, for the control of *Sclerotinia laxa* is indicated by the data from the 1943 and 1944 experiments. The chief object of the dormant eradicant spray under Wisconsin conditions is the suppression of the fungal sporodochia, though it doubtless retains some protectant fungicidal value at blossom time. The bloom spray is chiefly protectant in nature, but it has the further purpose of lessening the danger of spray injury from the dormant application. The possibility of using the bloom spray alone has been considered. This practice has been followed for many years, often with good results, in Europe and western North America. Unfortunately, however, under Wisconsin conditions a single spray application during bloom does not insure good control of *S. laxa* in epidemic seasons.

Suggested measures for the control of *Sclerotinia laxa* on sour cherry under conditions such as we have encountered are: (a) Apply a dormant tree spray of copper-lime-monocalcium arsenite plus fish oil, 3–2–2 (1½ pints fish oil)–50, shortly before budbreak, and (b) apply a spray of Bordeaux mixture, 3–4–50, soon after the blossoms have started to open.

SUMMARY

Many isolates of *Sclerotinia laxa* from sour cherry in Door County, Wisconsin, were grown in culture and compared with isolates obtained from various hosts from the Pacific Coast States. General colony characters were lobing, zonation, and scant conidial production.

Conidia formed in most isolates on Emperor grapes within 3 days at 16° to 24° C., or on potato-dextrose agar within 2 months at 4° to 8° C. Conidia germinated readily at 16° to 32° C. Germination occurred at 4° and 34.5°, but not at 37° C. The minimum time for germination was 2 hours. When sufficient nutrients were present, the germ tubes were crooked and branched.

The optimum temperature for radial growth on potato-dextrose agar was about 28° C. Some growth occurred at 4° and 32°, but none at 37° C.

The relationship of temperature to infection and disease development on small, potted, sour cherry trees was studied under controlled conditions in the greenhouse. Infection and blighting of inoculated blossoms were most rapid at 24° C., but occurred at 5° and 30° C. Eleven degrees centigrade was more favorable for spur blight development than 16° C. or higher.

Most blossom infections originated either through the anthers or the stigmas, although occasionally other blossom parts, especially petals, were attacked. Filaments and sepals were infected only through wounds or after contact with rotted tissue.

Blossom time was established as the critical period for infection, though under experimental conditions unopened, white-tipped buds were frequently infected. Infection of green fruits and leaves sometimes occurred under especially favorable conditions in the greenhouse and in the orchard.

No important difference in susceptibility between Early Richmond and Montmorency sour cherries was apparent in the greenhouse, although spur blight had been observed to be much more severe on Early Richmond in the orchard.

Experimental sprays for the control of *Sclerotinia laxa* were applied over a period of 3 years, and excellent control was obtained from certain programs. In the epidemic season of 1943 the eradicant spray of copper-lime-monocalcium arsenite plus cold-pressed menhaden fish oil, 3–2–2 (1½ pints fish oil) –50, applied just before budbreak, gave satisfactory suppression of sporodochia and 97 per cent reduction in incidence of spur blight. Ninety-nine per cent reduction of spur blight was obtained when this eradicant spray was followed early in bloom by a protectant spray of

Bordeaux mixture, 3–4–50. The bloom spray alone did not affect the production of sporodochia, but it reduced the incidence of spur blight 82 per cent.

In Wisconsin thorough applications of a dormant and a bloom spray are suggested for effective control of *Sclerotinia laxa* on sour cherry.

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A STUDY OF THE SUGAR BEET SEEDLING DISEASE IN OHIO

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INTRODUCTION

The black root disease of sugar beet seedlings is one of the most widespread and economically significant diseases of plants. It occurs wherever beets are produced on a commercial scale. Coons and Stewart (6), who have investigated the majority of the beet-producing regions of the United States, report yearly, national losses due to this disease ranging from a trace to 25 per cent of the planted acres. Buchholtz (2) writes that the disease constitutes the chief limiting factor in obtaining maximum stands and yields. Campbell (3) in 1940 reported the disease present in all fields he had examined in Washington, infection ranging from a trace to 95 per cent of the stand. He considers this disease to be one of the most important factors in sugar beet production.

In Ohio, black root of sugar beet seedlings is the major factor in determining the number of acres planted and harvested. The losses in this State closely approximate those reported by Coons and Stewart for the total sugar beet area.

Although the average annual loss to the state and national economy is great, the actual importance of this disease to the industry is best appreciated when it is realized that individual fields may have a 100 per cent loss in seedling stand with a consequent depressing effect on future acres planted. Despite the high cash value of the beet crop, the farmer is reluctant to plant his fields to beets when faced with the possibility of a complete loss of the crop. Labor is still another problem. Until black root can be controlled, hand labor will be required for the blocking and thinning operations since the machines used for these operations depend upon an almost perfect seedling stand.

In any root rotting disease, four possible means of control are available. First, the agents responsible for the disease may be eliminated from the soil in the immediate vicinity of the plant by the use of seed-borne fungicides or the soil may be treated directly with fungicidal or fungistatic agents. Second, soil amendments may be utilized to change the physicochemical nature of the soil, which may in turn affect the development of the disease. Third, varieties of the host plant may be developed which are resistant to infection. The fourth possibility, which may be related to the use of soil amendments, involves a change in the soil microflora with a resultant biologic antagonism between non-pathogenic soil organisms and those responsible for the disease.

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If any of these control measures is to be successfully developed it is first necessary to definitely establish the identity of the organisms causing the disease. Then investigations can be made of the physiology, morphology, and life cycle of the pathogen together with its relationship to the host plant and to other organisms of the soil.

Thus, this study was undertaken to determine the identity of the organisms responsible for the disease and the relative frequencies of their occurrence in diseased seedlings in Ohio.

A second objective of the work was to determine what effect, if any, applications of high phosphate content fertilizers had on the incidence of disease. Observations of commercial beet fields had indicated that in some instances the root rot disease was less severe in fields that had received high phosphate applications.

PERTINENT LITERATURE

The parasitic nature of this sugar beet seedling disease was first implied in 1890 when Hellriegel (15) found that by soaking the sugar beet seed ball in a one per cent solution of carbolic acid the amount of black root was significantly lessened. The knowledge regarding the organisms causing this disease was increased by Frank (12) and Kruger (17) who demonstrated that *Phoma betae* (Oud.) Fr. could cause a sugar beet seedling root rot. In 1901 Duggar and Stewart (8) isolated strains of *Rhizoctonia solani* Kühn from diseased sugar beet seedlings. In inoculation experiments with this organism the disease was reproduced in seedling beets. In 1906 Peters (33) described a seedling root rot caused by *Pythium debaryanum* Hesse and differentiated between the symptoms brought about by this organism and those due to infection by *Phoma betae*. Another of the agents of the disease was described in 1915 by Edson (9) when he isolated a previously undescribed fungus which he named *Rheosporangium aphanidermatus* (Edson) Fitz. and which was subsequently assigned to the genus *Pythium* and is now known as *Aphanomyces cochlioides* Drech.

The importance ascribed to these organisms as causal agents of the disease and to other fungi which have been considered responsible for black root is indicated by the following: Edgerton and Tims (10) have reported that *Rhizoctonia solani* infection caused losses up to 50 per cent of the crop and that *Phoma betae* was of minor importance in Louisiana. Campbell and others (4) isolated numerous fungi from beet seedlings in Washington but only *Phoma betae* isolates were pathogenic. Maxson (19) in isolations from 268 diseased seedlings found that *Fusarium* spp. constituted 53.36 per cent, *Phoma betae* 38.05 per cent, *Pythium* spp. 5.6 per cent, and *Rhizoctonia solani* 2.98 per cent of the isolations. In another series of observations he examined 970 diseased plants and found 70.93 per cent were infected with *Rhizoctonia solani*.

Reddy (21) has indicated that *Pythium debaryanum* was the principal cause of the disease while *Phoma betae* was of little importance. Buchholtz

(2) has reported that *Pythium debaryanum* caused at least 95 per cent of sugar beet seedling root rot damage. Coons, Kotila, and Bockstahler (5) have regarded *Aphanomyces* spp., common in all agricultural soils, as important causal agents of the disease. Melhus et al (20) found *Aphanomyces cochlioides* in only three of the fields examined by them. Hodges (16) isolated two species of *Fusarium* which he reported causing 50 to 75 per cent of all sugar beet seedling infections. From Europe, Esmarch (11) has reported *Phoma betae* as the primary cause of the disease with *Pythium debaryanum* ranking second and *Aphanomyces levis* DeBary third, the ratio of their occurrence being roughly 7–4–2. He also reported *Alternaria tenuis* Nees., and *Macrosporium cladosporioides* Desm. causing a seedling root rot. Greis (13, 14) has written that *Alternaria tenuis* spreads through the seed and attacks it saprophytically, later developing as a facultative parasite on the seedling. Marchal (18) has reported that the disease, in Belgium, is caused by *Bacillus mycoides* as well as by *Phoma betae, Pythium debaryanum,* and *Rhizoctonia solani.*

These various and, in some instances, contradictory reports concerning the fungi responsible for the disease and the relative importance of each of them as causative agents seemed to need clarification before an adequate control program could be developed for Ohio.

SYMPTOMS OF THE DISEASE

The black root disease of sugar beet seedlings has been divided into three distinct types. First, a preemergent type which, as the name implies, results in the death of the plants immediately after seed germination and prior to the emergence of the seedling from the soil. Second, a damping-off form of the disease which is apparent from the time of emergence of the seedling to the time when the first true leaves develop. In this type of the disease, the plants are attacked when the tissues are still rather tender and succulent. Infection of the beet seedling at this stage results in the complete wilting and immediate death of the plant. In many instances the disease develops so rapidly that the plant collapses at the base of the hypocotyl and falls over before the cotyledons wilt. A water-soaked, brown, discolored area extends up and down the hypocotyl or the upper portion of the young taproot from the point of entry of the pathogenic organism. In later stages this discoloration extends well up into the petioles of the cotyledons. The collapse of the hypocotyl of the seedling is followed within a very short time by the desiccation of the seedling. The hypocotyl of the seedling shrinks to an inconspicuous, dark, slender thread. The rapidity with which these events occur may be an explanation for the failure on the part of commercial growers in particular to recognize the large toll taken by this disease.

The third type of the disease is by far the most conspicuous of the three. The affected seedlings are not immediately killed and usually continue to live for several days after the initial symptoms appear. Fre-

quently such diseased plants recover and persist through the growing season. In these cases the tap root is replaced by numerous lateral roots. This lowers the quantity as well as the quality of the crop, because, although these lateral roots become fleshy, their total weight rarely equals that of a single normal beet root. The beets with numerous fleshy lateral roots have a lower sugar content and higher impurity content than do the larger single roots. The relatively high impurity content makes refining more difficult and the low sugar content naturally decreases the amount of recoverable sugar per ton of beets. This type of black root appears after the beets have developed beyond the early seedling stage. The beets that are infected after the first pair of true leaves are well developed frequently do not die. By this time secondary tissues have developed and prevent the complete destruction of the seedling. The hypocotyl or developing crown of the beet is composed essentially of intricately anastomosing vascular bundles containing lignified cells and is nearly free of parenchymatous cells (1). The name black root is particularly applicable to this type of the disease because of the general blackening of the hypocotyl which begins slightly below the ground and extends up into the cotyledons. The leaves of a plant so affected may develop an abnormal downward curvature but remain green and turgid.

METHODS AND MATERIALS

A majority of the diseased seedlings from which isolations were made were obtained from commercial fields although some were made from sugar beet seedlings growing in the greenhouse. Greenhouse seedlings were used for isolation work before field-planted beets were available and at various times during the summer when soil was removed from fields in which root rot was severe. The soil used in these studies was obtained from the beet-producing areas of the State.

The commercial fields from which specimens were collected were planted at irregular intervals from May 25 to July 1. Collections were made at weekly intervals beginning when only a few seedlings had emerged from the soil and continuing until the plants were well beyond the seedling stage.

The study of any plant disease is facilitated if the pathogens responsible for the disease can be isolated from the affected plant tissue by direct plating on a solid medium. For this to be successful it is either necessary to sterilize the surface of the diseased tissue without causing the death of any organisms within the tissue or to use a selective medium that will prevent or retard the growth of contaminating organisms. The environmental association of sugar beet roots with the fungi and bacteria of the soil make this goal difficult to attain. Many types and combinations of sterilizing agents were tested at varying concentrations and used in conjunction with various media. After numerous trials it was found that the sterilizing agents either killed all organisms present or failed to kill the nonpathogenic or saprophytic organisms associated with the beet root surfaces.

An additional difficulty was recognized when it became apparent that several organisms were involved, differing widely in characteristics and in susceptibility to sterilizing agents. Attempts to find a differential medium were equally unsatisfactory.

In order to obtain the cultures for this study a method described by Drechsler (7) was modified and used, with few exceptions, throughout the period during which diseased beets were collected.

Depending on the size of the seedling, the seedling without the leaves, the roots alone, or the hypocotyl, was washed thoroughly in tap water. The part used was then rinsed twice in sterile water and placed in approximately 20 cc. sterile tap water contained in a cotton-plugged tube. These were incubated for 24 to 48 hr. at room temperature then examined for the presence of mycelial growth surrounding the section of diseased seedling. All those on which such growth was not present were discarded. The sections surrounded by mycelial growth were removed from the tubes and blotted between folded pieces of sterile paper toweling. This removed the excess water and with it a large percentage of the bacterial contaminants. After blotting, the beet fragments were placed on corn meal agar medium in Petri dishes. After another incubation period of 24 to 48 hr. a section of agar was cut from the periphery of the developing colony. When this was transferred to potato-dextrose agar slants pure cultures would usually result.

Despite the superiority of this method of obtaining pure cultures it was considered possible that the actual relative numerical frequencies of the occurrence of the pathogens was not being shown by the isolation technique used. For this reason the following procedure was devised.

Collections of seedlings were made in the field by taking up the plants with the surrounding soil. A trowel was used to make a circular cut 6 to 8 inches into the soil at a distance of 2 to 3 inches from the beet. The plants were then lifted, an effort being made to keep the roots as nearly intact as possible. The plants and the adhering soil were wrapped in wax paper and taken to the laboratory where they were placed in water and the soil carefully removed from the roots. By doing this, comparatively few of the small lateral rootlets were broken. After the roots had been well cleaned they were floated in water in one half of a Petri dish and examined with the aid of a dissecting microscope. When lateral roots with some discoloration were observed they were removed in lengths of 3 to 5 cm. and placed in sterile tap water for additional washing. These fragments of roots were washed thoroughly in three changes of sterile distilled water and placed in a drop of sterile distilled water in the center of a vaseline-ringed cover slip. The cover slip was then inverted over a Van Tiegham cell mounted on a slide or over a deep, hollow ground slide. The completed preparations were placed in a large moist chamber and incubated at room temperature for 24 hr.

The growth characteristics of the organisms associated with the sugar beet seedling diseases in Ohio, as indicated from isolations thus made, were sufficiently distinctive to permit ready identification when the hanging drop preparations were examined microscopically.

In all pathogenicity tests sterile Brookston clay soil was mixed with cultures growing on the following medium: equal parts, by weight, of corn meal and sand were mixed, and the amount of the mixture that could be contained in a 100-cc. beaker was placed in a 200- or 300-cc. flask. One hundred ml. of water were then added to each flask. The flasks were plugged with cotton and autoclaved at 15 lb. pressure for two hours on two consecutive days.

Transfers were made from actively growing cultures of the fungi, on potato-dextrose agar slants, to the corn meal and sand medium. The mouths of the flasks were then covered with waxed paper to lessen the drying out of the medium and the cultures were incubated for 10 to 14 days. Following incubation the mycelium of the fungi had usually thoroughly permeated the medium. The entire mycelial culture was then removed from the flask and mixed into the soil at the rate of one such culture to two 6-inch pots of soil. After the soil was mixed with the culture it was well watered and left undisturbed for three days, after which time it was seeded with sheared seed of the type used in commercial plantings in Ohio.

The soil temperature studies were made in Wisconsin type temperature tanks.

RESULTS

Using the method of isolation previously described 689 cultures were obtained. The species distribution of the isolations is shown in table 1.

Because of the large number of isolates it was considered advisable to test pathogenicity of representative samples from each of the groups rather than test all of the isolates. In the preliminary test, any organism causing a reduction in the number of seedlings to emerge from the soil or any definitely diseased seedlings was selected for a second and more definitive test. In the preliminary test no attempt was made to reisolate the causative fungi from the diseased seedlings. Such reisolations were made from beets becoming diseased in the second test.

TABLE 1.—*Distribution of 689 isolations among species*

Organism	Times isolated	Percentage of total
1. *Aphanomyces cochlioides*	111	15.9
2. *Pythium debaryanum*	87	12.6
3. *Rhizoctonia solani*	52	7.6
4. *Alternaria tenuis*	22	3.2
5. *Fusarium* spp.	280	40.6
6. *Papulospora* sp.	30	4.3
7. Undetermined Phycomycetes	27	3.9
8. Unidentified	80	11.7

In all of the pathogenicity tests the soil was mixed with cultures of the fungi as previously described. The seed, sheared and untreated, was planted at the rate of 25 seed pieces to each 6-inch pot of soil.

After the seedlings had emerged from the soil they were examined frequently for indications of disease. The tests were culminated three weeks after seedling emergence. At that time counts were made of the apparently healthy plants and of the seedlings which had been removed because of disease. By totaling these two figures and comparing them with the number

TABLE 2.—*The pathogenicity of fungus isolates to sugar beet seedlings*[a]

Organism and isolate number	Plants emerging	Reduction in emergence based on mean of controls	Post emergence deaths	Plants alive at end of test	
Aphanomyces cochlioides	*Number*	*Number*	*Number*	*Number*	*Per cent*
1	12.0	14.6	12.0	00.0	00.0
2	13.0	13.6	13.0	00.0	00.0
3	15.5	11.1	15.5	00.0	00.0
4	12.5	14.1	1.0	11.5	43.2
5	17.5	9.1	6.0	11.5	43.2
6	18.0	8.6	3.5	14.5	54.5
7	18.0	8.6	3.0	15.0	56.3
8	19.5	7.1	3.0	16.5	62.0
9	19.0	7.6	2.0	17.0	63.9
10	23.5	3.1	1.0	22.5	84.5
Average	16.8	9.7	6.0	10.8	40.8
Pythium debaryanum					
1	4.5	22.1	1.5	3.0	11.2
2	4.5	22.1	0.0	4.5	16.9
3	9.5	17.1	3.5	6.0	22.5
4	10.5	16.1	1.5	9.0	33.8
5	16.5	10.1	2.0	14.5	54.5
6	16.0	10.6	4.5	11.5	43.2
7	19.0	7.6	1.5	17.5	65.7
8	24.5	2.1	6.5	18.0	67.6
Average	13.1	13.5	2.6	10.5	39.4
Rhizoctonia solani					
1	00.0	26.6	00.0	00.0	00.0
2	6.5	20.1	0.0	6.5	24.4
3	9.5	17.1	0.0	9.5	35.7
4	19.0	7.6	10.5	8.5	44.7
5	21.5	5.1	2.0	19.5	73.3
6	21.5	5.1	0.0	21.5	80.0
Average	13.0	13.6	2.0	10.9	43.0
Unidentified Phycomycetes					
1	5.0	21.6	2.5	2.5	9.3
2	15.0	11.6	5.0	10.0	37.5
3	22.5	4.1	5.0	17.5	65.7
4	27.5	−1.0	1.0	26.5	103.0
Average	17.5	9.6	3.3	14.1	53.9
Control	26.6	0.0	0.0	26.6	100.0

[a] Figures based on the average of two pots per isolate except for the control which is an average of eight pots.

of seedlings in the check pots the amount of preemergent disease could be determined.

Table 2 is a list of all isolates tested and their degrees of virulence. The results given are the averages from two inoculated pots for each isolate.

As would be expected, from the results of previous investigations, the various isolates of *Rhizoctonia solani* show varying degrees of virulence. This variation is undoubtedly related to the differing physiologic strains within the species. It is also illustrated by the results of this test that there are wide variations in virulence among the ten isolates of *Aphanomyces cochlioides* tested. A similar result is indicated for *Pythium debaryanum*, as well, but here the range or spread of pathogenicity is not so great as with *A. cochlioides*. In both instances it would seem that the differences between the least virulent and the most virulent are sufficiently great that

TABLE 3.—*Fungi identified from hanging drop cultures of sugar beet seedling tissues*

Date[a]	*Aphanomyces cochlioides*	*Pythium*	*Rhizoctonia*	*Fusarium*	Unidentified	Total
May 30	15	4	4	2	0	25
June 7	28	4	0	0	0	32
June 12	14	6	2	3	2	27
June 18	10	5	1	0	2	18
June 22	17	6	0	1	0	24
July 1	5	5	3	3	4	20
July 10	24	8	0	2	3	37
July 24	21	5	7	0	1	24
July 30	12	2	5	1	0	20
Total	146	45	22	12	12	210
Percentage of total	69	21	10	5	5	.....

[a] Approximate.

they can not be explained by the extremely slight environmental differences among the various units of this test.

Table 3 is a summation of the identifications made directly from the hanging drop preparations. No attempts were made to isolate pure cultures from these preparations.

Observations of field planted beets having indicated that high phosphate applications seemed to result in the reduction of the amount of black root in some plantings, one of the more virulent isolates of the *Aphanomyces cochlioides* was selected to study the effects of varying temperatures and phosphate applications on the development of the disease.

Sterilized Brookston clay soil contained in Wisconsin type temperature tanks was mixed with 10-day-old cultures of *Aphanomyces cochlioides* growing on potato-dextrose agar plates. The soil was well watered and left undisturbed for three days. It was then planted with seed of the type used in the pathogenicity tests and phosphate was added at the rates of 400 and 500

lb. super phosphate per acre. There were two containers of each treatment at each temperature and two check containers (no fertilizer) at each of the five temperature levels used in the test. The averaged results are in table 4.

TABLE 4.—*Effect of temperature and phosphate applications on the development of black root of sugar beet seedlings*

Temperature in degrees C.	Percentage of beets dead in relation to the total number emerged in checks		
	No fertilizer	(Super-phos. 400 lbs.)	(Super-phos. 500 lbs.)
32	89.5	44.9	86.4
28	58.5	86.1	74.4
24	97.0	78.4	64.0
20	66.6	12.0	30.9
18	50.0	8.1	11.3

SUMMARY AND CONCLUSIONS

Based on the number of times isolated, the fungus primarily responsible for black root of sugar beets, in Ohio, is *Aphanomyces cochlioides* Drech., the isolates of this fungus constituting 15.9 per cent of a total 689 isolations. This fact was amply substantiated by observations made by identifying the organism in hanging drop preparations. Of 210 identifications made in this way 69 per cent were *Aphanomyces cochlioides*. *Pythium debaryanum* Hesse appeared in culture with a frequency almost equal to that of *Aphanomyces cochlioides* but in the hanging drop preparations the latter was identified 146 times to 45 times for *Pythium debaryanum*. Third in frequency of isolation and identification as a pathogen was *Rhizoctonia solani* Kühn: 7.6 per cent of the isolations and 10 per cent of the hanging drop cultures were identified as this species. Some of the isolates of *Rhizoctonia solani* were among the most virulent pathogens.

Seasonal variation in the organisms isolated was negligible. During the season the ratio of one fungus isolated to another was remarkably constant.

From the temperature tank phosphate experiment it can be concluded that the amount of black root due to *Aphanomyces cochlioides* tends to increase when soil temperatures rise from 18° to 24° C. and are higher despite phosphate applications at temperatures of 24°, 28°, and 32° than at 20° and 18° C.

At 28° and 32° C., phosphate applications have no effect in reducing the amount of black root. However, at soil temperatures of 18°, 20°, and 24°, both 400- and 500-lb. applications caused a marked reduction in the number of seedlings becoming diseased.

This fertilizer effect at low temperatures is highly significant from a practical standpoint because these are the temperatures that prevail during the early spring when most of the beet acreage is being planted.

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A DISEASE OF CABBAGE AND OTHER CRUCIFERS DUE TO CERCOSPORELLA BRASSICAE[1]

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During the late winter and spring of 1943–1944 the foliage of cabbage being grown for seed in Coos and Curry Counties, Oregon, was infected with a *Cercosporella* sp. hitherto unreported on this host in Oregon. Bird rape (*Brassica campestris* L.), known locally as wild mustard, and turnip (*Brassica rapa* L.) growing close by were found abundantly infected with the same or a closely related organism.

A brief description of this disease on cabbage in Oregon has already been published in a preliminary report (15).

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Leaf spots of *Brassica* spp. due to Cercospora-like fungi are widely distributed. They have been reported from the following countries: Brazil (22), England (5, 7, 20, 22), France (22), Guam (22), Puerto Rico (25, 26), China (13), United States (1, 25), and Yugoslavia (22). In the United States, leaf spots of *Brassica* spp. due to Cercospora-like fungi have been reported from the following states: Alabama (25), California (11, 21, 25), Connecticut (1, 25), Delaware (25), Florida (1, 24, 25), Georgia (25), Illinois (1, 25), Indiana (3, 25), Louisiana (2, 25), Massachusetts (8, 25), Maryland (25), Minnesota (25), Mississippi (17, 25), Missouri (14, 25), New Hampshire (25), New Jersey (25), New York (25), Oklahoma (25), Oregon (6, 15, 25), Pennsylvania (16, 25), South Carolina (25), Texas (1, 12, 25), and Virginia (25).

The disease on cabbage has so far been reported only from the following states: Alabama (25), California (21), Mississippi (17), and Oregon (15). It also has been reported from Puerto Rico (25, 26).

ECONOMIC IMPORTANCE OF THE DISEASE

The disease on cabbage has not been of major importance in Oregon. Infected plants have not been damaged severely, as the disease was confined to the lower leaves. It did not attack the inflorescences. However, the causal fungus has caused economic damage to mustard and turnip being

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FIG. 1. Young lesions on a cabbage leaf caused by *Cercosporella brassicae*. Note dendritic type of lesion.

grown for greens in other regions (2, 24). Moreover, damage to cultivated turnip and mustard plants being grown for seed production has resulted from a reduction in the functional leaf area.

SYMPTOMS

Cabbage. Only the leaves of the cabbage plant have been attacked by this fungus. In its initial stages, the disease superficially resembles downy mildew of cabbage due to *Peronospora parasitica*, the young lesions being small, averaging approximately 2 mm. in diameter, dark gray or black, and dendritic in form (Fig. 1). The lesions later lose their dendritic configuration and become more or less rectangular or rounded with well-defined margins. The mature lesions measure from 10 to 22 mm. in diameter and are ashy black.

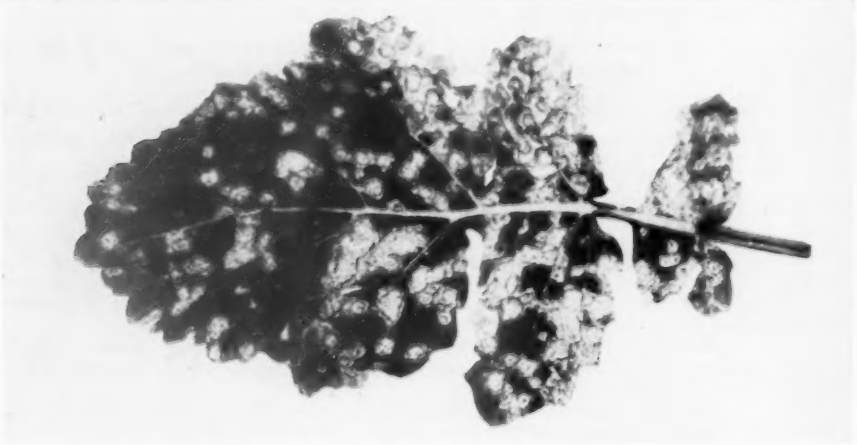

FIG. 2. Lesions on turnip leaf caused by *Cercosporella brassicae*.

Turnip and wild mustard. The lesions on turnip and wild mustard leaves are conspicuous, typically round, semi-transparent, with brownish-gray centers and well-defined brown or tan margins (Fig. 2); they are larger than those on cabbage, averaging about 30 mm. in diameter when fully developed. An extreme necrosis of the invaded tissues occurs. The lesions do not have the dendritic form so characteristic of the disease in its initial stages on cabbage.

PATHOLOGICAL HISTOLOGY

The fungus mycelium in both cabbage and turnip leaves is predominately intercellular.

In turnip, the protoplasts and cell walls in the invaded areas are destroyed which results in a necrotic type of lesion. In cabbage, however, killing of the cells is more restricted. The paths of the lateral hyphae are demarked by black, necrotic masses formed from the middle lamella and abutting cell wall (Fig. 3, C). The adjacent cells are partly emptied, but the protoplasts of those farther away from the advancing hyphae are only yellowed with masses of lipoid elements present as free oils or held in large vacuolate elaioplasts. The localization of killed areas accounts for the dendritic pattern of the young lesions.

THE CAUSAL ORGANISM

Morphology

The conidia of the causal fungus from lesions on cabbage vary at maturity from 70 to 100 $\mu \times 2$–3 μ, the average dimensions being 80 $\mu \times 2.5$ μ. They are hyaline, elongate-cylindrical, mostly 3- to 4-celled, with indistinct cross walls. The young conidia are more or less straight but mature conidia are typically curved at both ends (Fig. 3, A). The conidia are not borne on a stroma but originate from hyphal strands loosely aggregated beneath the stomata. They are commonly borne uniformly over the surface of the lesion.

Conidia from lesions on wild mustard and turnip are similar to those of the fungus on cabbage except that they are irregularly partitioned, thus having one cell of smaller dimensions than the others. On freshly collected material, the conidia contain from 3 to 5 cells and average 65 $\mu \times 2.5$ μ (Fig. 3, B). As in the lesions on cabbage, the conidia are not borne from a stroma but from hyphal strands which generally are massed within the stomatal openings.

Taxonomy

From its conidial characteristics the causal fungus is evidently *Cercosporella brassicae* (Fautr. et Roum). Hoehnel (18, 23), a synonym for which is *Cercospora* (*Cercosporella*) *albo-maculans* Ell. et Ev. (9, 19).

Host Range and Pathological Significance

Cercospora-like fungi have been reported on the following Crucifers: *Brassica oleraceae* var. *capitata* L. (1, 13, 21, 25), *B. oleraceae* var. *italica*

Plenck. (3, 14, 25), *B. oleraceae* var. *viridis* L. (6, 25), *B. chinensis* L. (11, 22), *B. campestris* L. (8, 13, 25), *B. juncae* Coss. (25), *B. nigra* L. Koch (25), *B. napus* L. (25, 26), *B. kaber* (D. C.) L. C. Wheeler (25), *B. oleracea* var. *gemifera* D. C. (11), *B. campestris* var. *napobrassica* (L.) D. C. (18,

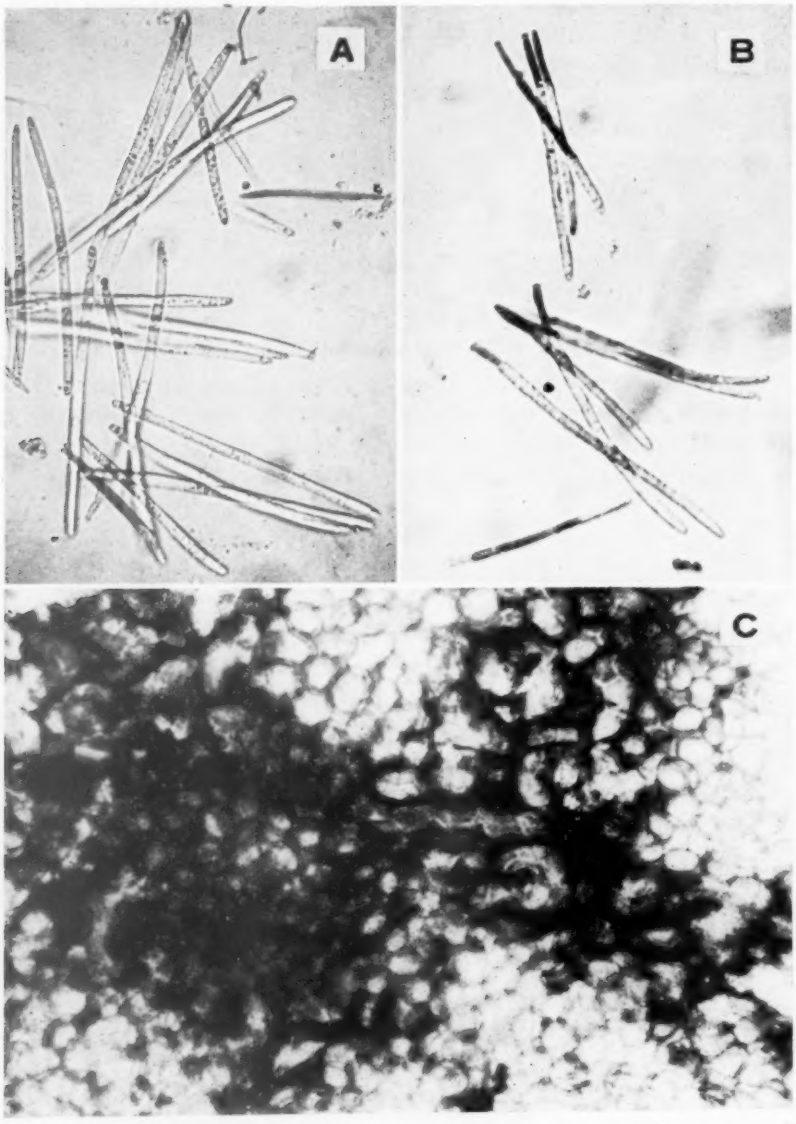

FIG. 3. A and B, Conidia of *Cercosporella brassicae:* A, from lesions on cabbage leaf, ×200; B, from lesions on turnip leaf, ×200; C, Photomicrograph of young lesion on cabbage leaf showing the effect of the destruction of cell walls, ×75.

25), *B. pekinensis* (Lour.) Rupr. (1, 25), *B. rapa* L. (1, 24, 25, 26), and *Raphanus sativus* L. (4, 10, 14, 25).

Despite the widespread occurrence of Cercospora-like fungi on many Crucifers, there are relatively few reports of these fungi causing economic

damage. In 1927, Davis (8) tried to infect beets, celery, parsnips, carrots, and cabbage by inoculation with conidia of a Cercospora-like fungus from lesions on Chinese cabbage but without success. Weber (24) reported that in Florida *Cercosporella albo-maculans* was widespread and common on turnip, but not severe enough to cause any damage except in certain instances when it prevented turnip greens from being marketed. Chupp, in a personal communication, stated that the fungus was rather prevalent and sometimes destructive on turnip in New York. In 1943, Snyder and Baker (21) reported a leaf spot on cabbage in California due to *Cercospora* sp. but the disease was of little economic significance, being confined to the lowest leaves on the plant. In 1944, Baines (3) reported leaves of broccoli (*Brassica oleracea* var. *italica*) were severely infected with *Cercosporella albo-maculans* (E. & E.) Sacc.

Cross Infection Studies

The occurrence of this disease on cabbage, wild mustard, and turnip growing in close proximity suggested that wild mustard or turnip was the likely source of the disease on cabbage. To determine this, cross inoculations under controlled greenhouse conditions were performed using conidial suspensions from sporulating lesions on cabbage and turnip, respectively. This procedure was necessitated by the failure of the causal organism to fruit in culture. Numerous lesions indistinguishable from those on turnip in nature were produced on leaves of young turnip plants by atomizing them with suspensions of conidia from sporulating lesions on cabbage. Conversely, typical lesions were produced on leaves of young cabbage by atomizing them with suspensions of conidia from sporulating lesions on turnip. These results indicate that the disease on these two hosts has a common cause.

SUMMARY

A disease of cabbage and other Crucifers caused by the fungus *Cercosporella brassicae* (Fautr. et Roum.) Hoehnel is described and discussed.

The geographical distribution, economic importance, and host range of the disease are discussed.

The causal fungus and its effects on host cells are described.

Cross-inoculation studies indicate that the disease on cabbage, wild mustard, and turnip has a common cause.

U. S. DEPARTMENT OF AGRICULTURE

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AN ANTIBIOTIC SUBSTANCE ACTIVE AGAINST CERTAIN PHYTOPATHOGENS [1]

CURT LEBEN AND G. W. KEITT [2]

(Accepted for publication June 1, 1948)

During the past few years the marked advances in the use of antibiotic substances in the field of animal disease control have stimulated research on the use of these agents in the control of plant diseases incited by bacteria or fungi (e.g., 1, 2, 4, 6, 7, 8, 9, 10, 13, 15, 16). In this connection, this laboratory has published notes on an unidentified antibiotic from an actinomycete, which was effective as a protectant spray against apple scab and tomato early blight in greenhouse tests (11, 12). The purpose of the present paper is to report more fully studies on the production, purification, properties, and *in vitro* biological activity of this material. Investigations on its possible use in the control of plant diseases are being extended and will be reported later.

The antibiotic has tentatively been given a name, antimycin, since on the basis of properties and biological effects described in the present paper it appears to be distinct from all others that have come to our attention.

THE ANTIBIOTIC ORGANISM

In 1945, a plate culture of *Venturia inaequalis* (Cke.) Wint. was markedly inhibited by a white, slow-growing actinomycete contaminant. This organism produced the musty odor typical of actinomycetes, and in old potato agar[3] cultures a small amount of a green-black pigment was noted. According to the classification of Waksman and Henrici (17) the organism has been placed as an undetermined species in the genus *Streptomyces* (See figure 1, A).

Several modes of culture have been used to grow the antibiotic organism, and in each antimycin was produced. Temperatures employed in the various experiments ranged from 24° to 28° C. On nutrient agar or potato agar, growth was satisfactory and the active substance readily diffused through the medium. In continuously shaken liquid substrates such as a corn steep liquor medium[4] the organism grew vigorously as a heavy suspension of spores and hyphae. The maximum antimycin concentration in the culture filtrate for this method of growth was reached in three or four days. During this time there was a reaction change from pH 7.0 to pH 8.0–8.5.

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[2] Research associate and professor, respectively. The authors are indebted to Roland Hodgson, John E. Mitchell, and W. H. Peterson for their aid and suggestions, and to Eugene Herrling, who prepared the photographs.

[3] Per liter: extract from 40 gm. of sliced, peeled potatoes steamed one half hour in 500 ml. of water; glucose, 5 gm.; agar, 17 gm.

[4] Per liter: corn steep liquor (50 per cent solids), 50 gm.; glucose, 10 gm.; and $CaCO_3$, 1.5 gm. The medium was adjusted to pH 7.0 before the $CaCO_3$ was added.

The organism also has been grown in 14-liter lots of corn steep liquor meddium in stationary tanks provided with stirrers and means for continously passing sterile air through the medium.[5] Shake flask fermentations have given yields of 1–3 units (a unit is defined later in this paper) per ml. of the culture filtrate; in tank fermentations yields of 3–5 units per ml. have been obtained.

The organism has been kept for over two years in soil or under oil (14) with apparent stability.

ASSAY, PARTIAL PURIFICATION, AND SOME PROPERTIES OF THE ACTIVE MATERIAL

A blotting paper disc assay using the fungus *Glomerella cingulata* (Stoneman) Spauld, and v. Schrenk., our isolate number 22, was employed to determine the potency of antimycin in various preparations. In preliminary tests this isolate gave the most satisfactory type of inhibition rings in trials using various temperatures, media, and a number of fungi that were inhibited by the culture filtrate. For the assay, 20-ml. amounts of nutrient agar were allowed to harden in Petri plates. These were then layered with five ml. of a spore suspension of the assay organism in warm (40°–45° C.) nutrient agar. Blotting paper discs 6 mm. in diameter were dipped in the preparation to be assayed, drained in a uniform manner, placed on the surface of each of three seeded plates, and the plates incubated 24 to 36 hours at 28° C. On each plate usually five different test preparations and a standard solution of one unit per ml.[6] were tested at one time. The diameters of the resulting zones of inhibition were measured and the potency of a given preparation determined with the aid of a dilution curve of the standard. The diameter of the zone of inhibition for a given preparation is a straight line function of the log of the concentration. The assay has not been evaluated statistically; however, we have found it useful for the type of studies that will be described.

The antibiotic in the culture filtrate was partly purified by means of the method indicated in table 1. Cultures were prepared by heavily seeding 300-ml. amounts of the corn steep liquor medium in liter Erlenmeyer flasks, which were shaken in a reciprocating shaker for four days at 26° C. Antimycin has also been extracted from the culture filtrate with butanol, although in our hands this method was not so efficient as that given in table 1.

Ethanol solutions prepared as indicated in table 1 were deep red and had the earthy odor typical of actinomycetes. On the addition of four to nine volumes of water, an amorphous precipitate was formed that contained all, or nearly all, of the activity. From this precipitate antimycin was extracted with water at pH 9.3 and partly extracted with water at

[5] For these tests the tank fermentation equipment of the Department of Biochemistry, University of Wisconsin, was used. Grateful acknowledgment is made to M. J. Johnson and W. E. Brown for aid and suggestions made in connection with this phase of the work.

[6] An ethanol solution of the active material, giving zones of inhibition from 16 to 18 mm., was arbitrarily assigned a potency of one unit per ml. and used as a standard. It has been kept 14 months at 8° C. with no apparent loss in activity.

pH 6.0; it was reprecipitated in an amorphous form by adjusting the pH to 2.5. At pH 9.3 no activity remained after two days at room temperature;

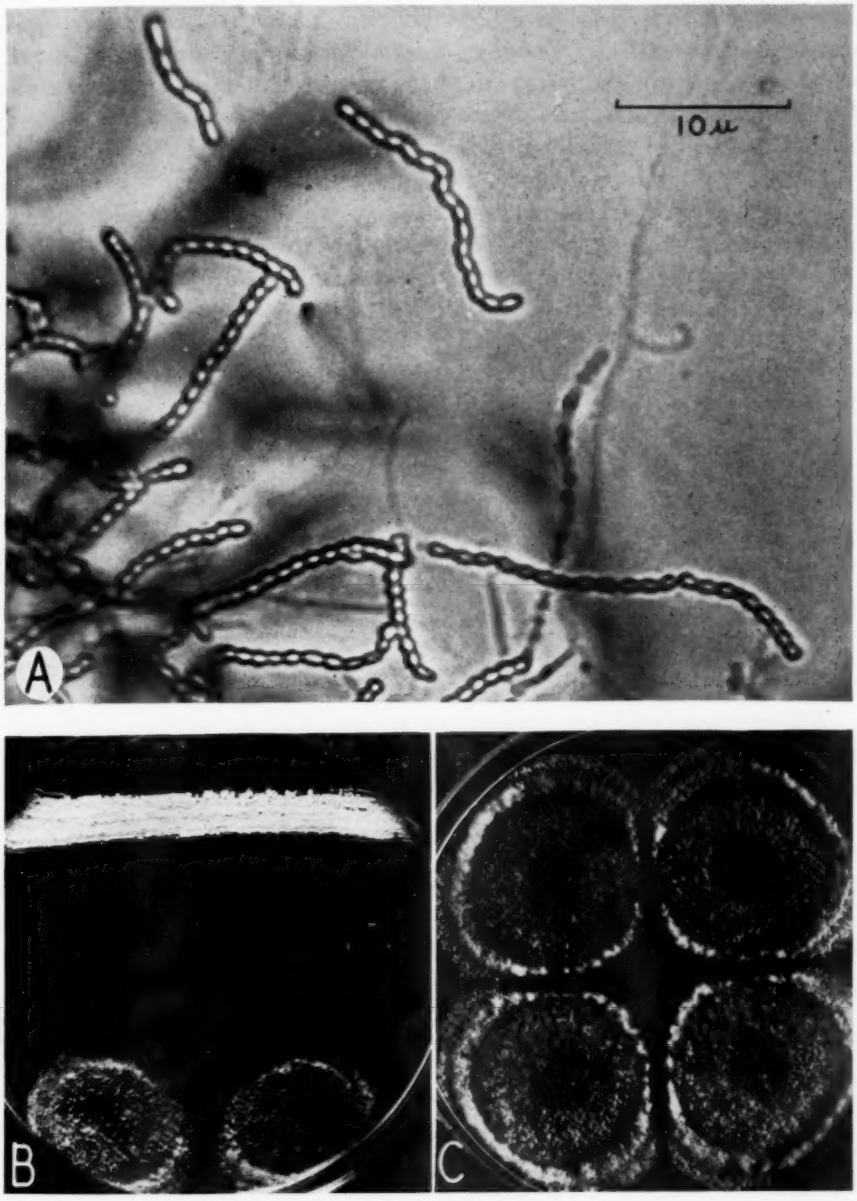

FIG. 1. A. Photomicrograph of aerial conidial chains of the antibiotic organism. B. *Sclerotinia fructicola* (Wint.) Rehm seeded 5, 20, and 35 mm. from a streak of the antibiotic organism. Note complete inhibition of *S. fructicola* at 5 mm. and retarded growth at 20 and 35 mm. C. Control plate containing *S. fructicola*.

at pH 11.0 no activity remained at the end of an hour. Antimycin in the culture filtrate (pH 8.3) was inactivated in eight minutes at 100° C. Water

suspensions at pH 2.5 and ethanol solutions appeared to be stable at 8°C. About one half of the activity in ethanol solutions was lost on refluxing 1.5 hours.

Antimycin was adsorbed by Norite from ethanol and from the culture filtrate; elutions with a number of reagents were not successful. Dialyzability was tested by sealing 25 ml. of the culture filtrate in a Visking cellophane bag, which was suspended overnight in a covered beaker containing 500 ml. of distilled water and a few drops of chloroform to act as a preservative. An assay indicated no change in potency of the residue in the bag; the dialysate, when concentrated to a small volume under reduced pressure, showed no activity. Aqueous suspensions at pH 6.1 (15 units per ml.) gave the following negative tests: Molisch, biuret, Hopkins-Cole, xanthoproteic, Millon, $FeCl_3$, and ninhydrin.

TABLE 1.—*Fractionation procedure*

Step	Treatment	Total volume	Total units	Units per mg. dry weight
		ml.		
I.	Culture centrifuged. Sediment discarded.	800	[a]	0.16
II.	Supernatant acidified to pH 2.5 (HCl). Suspension centrifuged. Inactive supernatant discarded. Precipitate extracted four times with 40-ml. amounts of absolute ethanol.	160	2,400	7.57

[a] Not assayed directly. Assumed to be 2,400 units since discarded supernatant in step II. was inactive.

When ethanol solutions prepared as indicated in table 1 were dried *in vacuo* and from one to four times the original volume of various solvents added and allowed to stand four to 12 hours, the maximum activity that could be accounted for in the various solvents was from 30 to 50 per cent of the original. Under these conditions reagents that dissolved the maximum amount of antimycin were acetone, butanol, chloroform, ethanol, ether, ethylene glycol, methanol, methyl acetate, propylene glycol, and pyridine. Antimycin was less soluble in benzene, carbon tetrachloride, petroleum ether, and toluene, and insoluble in water.

Fractionations have been carried further than indicated in table 1. These procedures, at present not fully evaluated, are being studied further and will be reported later.

THE ACTIVITY OF ANTIMYCIN IN VITRO

Screening Tests

In screening tests, the antibiotic organism was streaked near the side of plates containing nutrient agar (for bacteria) or potato agar (for fungi), and the plates incubated four days at 24° C. to permit growth of the organism and the diffusion of antimycin. Plates then were seeded with test organisms. Bacteria were streaked perpendicularly from the *Streptomyces*

culture, and fungi were seeded with small pieces of the mycelium placed at varying distances from the *Streptomyces* streak[7]. Inhibition was noted in two or more trials by the absence or reduction of growth adjacent to the antibiotic organism after an incubation period at a temperature suitable for the growth of the test organism (Fig. 1; B, C). All of the fungi except *Venturia inaequalis* were incubated four days at 24° C.; *V. inaequalis* was incubated 2 weeks at 16° C. The phytopathogenic bacteria were incubated two days at 24° C.; the remaining bacteria were incubated 24 hours at 37° C.

All the fungi tried in the screening tests were inhibited in varying degrees. The species tested were: *Alternaria solani* (E. and M.) Jones and Grout, *Ascochyta* sp. (from pea)[8], *Chalara quercina* Henry, *Cochliobolus* (*Ophiobolus*) *miyabeanus* Ito and Kuribay, *Colletotrichum circinans* (Berk.) Vogl., *C. graminicolum* (Ces.) G. W. Wils.[8], *C. lagenarium* (Pass.) E. and H., *C. lindemuthianum* (S. and M.) Bri. and Cav.[8], *C. pisi* Pat., *C. phomoides* (Sacc.) Chester, *Fusarium oxysporum* f. *conglutinans* (Wr.) S. and H., *F. oxysporum* f. *lycopersici* (Sacc.) S. and H., *F. oxysporum* f. *nicotianae* (Johns.) S. and H., *F. oxysporum* f. *pisi* (Sny.) S. and H., *Gibberella zeae* (Schw.) Petch, *Glomerella cingulata*[8], *Helminthosporium sativum* Pam., King, and Bakke, *Macrosporium* sp. (from muskmelon), *M.* sp. (from pea), *Mycosphaerella citrullina* (Sm.) Gross., *Neurospora crassa* Shear and Dodge, *Nigrospora sphaerica* (Sacc.) Mason, *Phoma lingam* (Tode) Desm., *P. terrestris* Hansen, *Phycomyces blakesleeanus* Burgeff, *Pythium graminicolum* Subr., *P.* sp., *Sclerotinia fructicola*, *S. laxa* Ader. and Ruhl., *S. minor* Jagger, *S. sclerotiorum* (Lib.) De Bary, *Stemphylium sarcinaeforme* (Cav.) Wilts., and *Venturia inaequalis*[8].

The bacteria used in screening tests were the following: *Agrobacterium tumefaciens* (Smith and Townsend) Conn, *Bacillus cereus* var. *mycoides* (Flügge) Bergey et al., *B. subtilis* Cohn *emend* Prazmowski, *Corynebacterium fascians* (Tilford) Dowson, *Erwinia amylovora* (Burrill) Winslow et al., *Escherichia coli* H 52 (Migula) Castellani and Chalmers, *Micrococcus pyogenes* var. *aureus* 209 P. (Rosenbach) Zopf, *Proteus vulgaris* Hauser, *Pseudomonas aeruginosa* (Schroeter) Migula, *P. savastanoi* (Erw. Smith) Stevens, *P. solanacearum* Erw. Smith, *Serratia marcescens* Bizio, *Xanthomonas beticola* (Smith, Brown, and Townsend) Bergey et al., and *X. campestris* (Pammel) Dowson. The only species that were affected were *B. cereus* var. *mycoides* and *E. amylovora*, which were completely inhibited 12 and 17 mm., respectively, from the *Streptomyces* culture.

Agar-streak Tests

With some of the organisms found to be inhibited in the screening tests, agar-streak tests (18) were made to determine if the material partly puri-

[7] For screening tests and the agar-streak tests, which are described later, inocula of all fungi except *Venturia inaequalis* were prepared from potato agar cultures that had grown four to seven days at 24° C. That for *V. inaequalis* was made from three- to five-week cultures kept at 16° C. Suspensions of the phytopathogenic bacteria were made from nutrient agar cultures that had grown 48 hours at 24° C.; the suspensions for the remaining bacteria were prepared from 24-hour cultures held at 37° C.

[8] More than one isolate tested.

fied on the basis of results with one assay organism was effective against other organisms, and, if so, to note the amount of the antibiotic substance necessary to inhibit the various organisms.

From a concentrate prepared as indicated in table 1, dilutions were made in sterile water. The resulting suspensions were mixed well in warm (40°–45° C.) potato agar, plates were poured, and the medium was allowed to solidify. Media containing the same amounts of ethanol as in dilutions

TABLE 2.—*The concentration of an antimycin preparation that prevented growth of various organisms in agar-streak tests*

Organism[a]	Isolate number	Inhibition point[b]
		µg. per ml.
Ascochyta sp. (pea)	....	25.0
Bacillus cereus var. *mycoides*	157	125.0
Do	158	125.0
Cheilara quercina	....	0.8
Colletotrichum circinans	....	1.6
Do *lindemuthianum*	....	12.5
Do *phomoides*	....	0.8
Do *pisi*	....	1.6
Fusarium oxysporum f. *lycopersici*	....	> 250.0
Do *nicotianae*	....	> 250.0
Do *pisi*	....	> 250.0
Glomerella cingulata	3	0.8
Do	16	1.6
Do	18	3.1
Do	22	6.3
Do	28	62.5
Nigrospora sphaerica	....	0.2
Phoma lingam	....	0.4
Pythium sp.[c]	....	250.0
Sclerotinia fructicola	12	1.6
Do	118	0.4
Stemphylium sarcinaeforme	....	1.6
Venturia inaequalis	365–4	0.8[d]
Do	454–8	0.8[d]
Do	637–2	0.4[d]
Do	898–4	0.4[d]

[a] All fungi except *V. inaequalis* were incubated two days at 24° C. before data were taken; *V. inaequalis* was incubated two weeks at 16° C. *B. cereus* var. *mycoides* was incubated 24 hours at 37° C. before data were taken.

[b] The smallest amount of the antimycin preparation (dry weight) that completely inhibited growth.

[c] Streaked with a suspension of mycelial fragments.

[d] Results based on one trial.

of the antimycin preparation were used as controls[9]. Plates were streaked with the test organisms, incubated at a temperature suitable for the growth of these organisms, and noted for inhibition when growth became clearly visible on control plates.

A summary of the data is given in table 2. Entries are based on two or more trials, except as noted. The growth of those species that were not completely inhibited at the highest concentrations used was markedly re-

[9] The maximum ethanol content of test and control media was one per cent.

tarded. Amounts smaller than indicated in table 2 have been reported (11) to inhibit *Sclerotinia fructicola* and *Venturia inaequalis;* those data were obtained, however, with a preparation more purified than that used in the present studies.

The end points given in table 2 are for the conditions noted; under other conditions different end points were obtained. For example, if instead of fungus spores, small fragments of mycelium were used in seeding, much greater concentrations were needed to prevent growth. Incubation time was also important. The end point for *Nigrospora sphaerica* was found to be 0.2 μg. per ml. at the end of a two-day incubation period; at the end of ten days, however, the end point was 1.6 μg. per ml. Probably many other factors are important in this connection.

DISCUSSION

The present paper has reported studies on the production, partial purification, properties, and *in vitro* biological activity of an antibiotic substance produced by an unidentified species of *Streptomyces.* This material, like viridin (3) and glutinosin (5), appears to be more generally effective against fungi than bacteria. While most of the fungi dealt with in the present paper are phytopathogens, recent studies, as yet incomplete, indicate that certain fungi pathogenic to humans are also inhibited by the antibiotic.

A partially purified material, which represented a 47-fold increase in activity over the culture filtrate, had an inhibitory effect on all of the organisms tested that were antagonized in the presence of the growing antibiotic organism. There were, however, marked differences in the amounts of this material necessary to inhibit different species and even different isolates of a single species. The order of this sensitivity was in a general way parallel to that observed in the presence of the growing antibiotic organism.

SUMMARY

A species of *Streptomyces* was antagonistic on agar to all of 33 fungi tested. It was not antagonistic on agar to a number of bacteria, including several species commonly used in antibiotic assays. The antibiotic, which is tentatively named antimycin, was produced in shake flasks and by tank fermentation. It was partly purified by ethanol extraction of the precipitate formed when the culture filtrate was adjusted to pH 2.5. The active material was nondialyzable, heat labile, and soluble in a number of organic solvents and in water at pH 9.3. Water solutions at pH 9.3 became inactive in a few days; water suspensions at pH 2.5 and ethanol solutions appeared to be stable. In agar-streak tests, the partly purified material prevented or reduced the growth of *Bacillus cereus* var. *mycoides* and all of the 16 fungi tested. The most sensitive organism, *Nigrospora sphaerica,* was completely inhibited by 0.2 μg. per ml.; several test organisms were

not completely inhibited by 250 μg. per ml., the highest concentration employed.

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STUDIES ON THE BACTERIOPHAGE OF XANTHOMONAS PRUNI

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INTRODUCTION

Studies on the bacteriophage of *Xanthomonas pruni* (E. F. Smith) Starr and Burkholder, were undertaken to expand the knowledge on this bacterial virus in light of the techniques, equipment, and concepts that have become available since the isolation of the phage in 1927 (1). Since this bacteriophage is among the first bacterial viruses recognized for plant pathogenic bacteria, its properties, in addition to current interest, are of historical significance. The purpose of this paper is to give the results (12) and to discuss the utility and implication of the findings.

METHODS AND STUDIES

The bacteriophage, when originally isolated and concentrated in 1927, was preserved in sealed glass tubes. In May, 1947, after 20 years of storage in darkness at room temperature, the phage at 10^{-1} dilution lysed young cultures of *Xanthomonas pruni* upon its initial addition to the culture.

Increase of phage titer was accomplished by shallow-broth culture which permitted dense growth of the bacteria. Rectangular two-quart milk bottles containing 100 ml. of sterilized liquid culture medium[3] were placed on their broad side after inoculation and at 22°–24° C. for 24 hr. The broth within the bottles was approximately 0.5 cm. deep and presented an exposed surface of about 2 cm.^{2} per ml. Sufficient solution of oxygen was secured by these conditions to permit dense growth of the bacterium throughout the medium. Lysis of such cultures yielded phage with a titer of 10^{-9} after Seitz filtration for sterilization and removal of bacterial fragments and sediments. Twenty-ml. aliquots of this phage filtrate were stored in 25-ml. glass serum bottles with rubber stoppers for further studies. The titer of the originally isolated phage was 10^{-5} when stored. It was not determined when revived. Its maximal increase by 12 serial passages through 24 hours broth cultures 3.5 cm deep (5 ml. of broth per 15×150 mm. tube) was 10^{-7}. With shallow-broth culture the titer was increased from 10^{-7} to 10^{-9} in one lysis procedure.

Specificity of the phage was evident from phage inoculations to cultures of isolates of *Xanthomonas pruni* and of other plant pathogenic bacterial species. All isolates of *X. pruni* from peaches, plums, and apricots located

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[3] Beef extract 3 gm., peptone 5 gm., glucose 2 gm., NaCl 5 gm. in one liter of distilled water adjusted to pH 7 5 for pH 7.2–7.3 after sterilization

in various regions of Illinois (vicinities of Belleville, Carbondale, Centralia, Olney, and Urbana) were lysed by the phage. Other species of bacteria tested (*Erwinia amylovora* (Burrill) Winslow et al, *X. campestris* var. *armoraciae* (McCulloch) Starr and Burkholder, and *X. lactucae-scariolae* (Thornberry and Anderson) Burkholder (See 11)), the last distinguishable from *X. pruni* only by pathogenicity, were not lysed. The lack of lysis in a culture of *E. amylovora* isolated from apricot twigs which is a host for lysable *X. pruni* indicates that the host in which the organism is parasitic either does not contribute to the specificity of lysis or such determinative characteristic is readily lost during multiplication and growth in the artificial medium used for isolation and perpetuation of the pathogen.

Electron microscopy[4] was employed to disclose the morphology of any

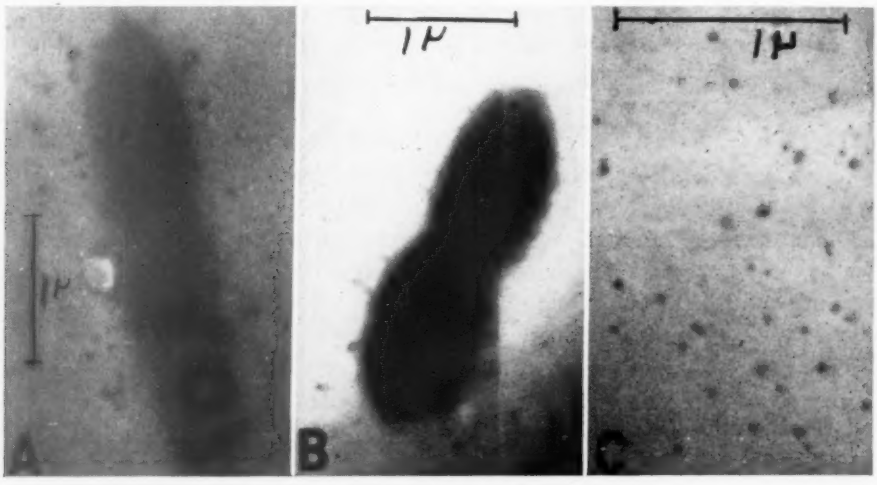

FIG. 1. Electron micrographs of *Xanthamonas pruni* bacteriophage particles (A) attached to a single cell, (B) attached to a constricted cell, (C) in suspension.

particles similar to those recognized as other bacteriophages and the adsorption of these particles by the host bacteria. The bacteriophage in a filrate of 10^{-9} titer was added to a suspension of growing cells of *Xanthomonas pruni* and then observed under the electron microscope (Type B, RCA). The bacteria were surrounded with small spherical particles, approximately 50 mμ (millimicrons) in diameter (Fig. 1). Electron micrographs of the phage filtrate revealed similar particles (Fig. 1). In very few cases, there was a suggestion that these particles possessed a "tail" or "handle" structure about 10 by 100 mμ. However, a "spherical" rather than a "tadpole" shape (particle with appendage) seemed to be characteristic of these particles. In this respect, the particles resembled the T3 and T7 bacteriophage of *Escherichia coli* (2), which are small spheres of 45mμ diameter. Also like the T3 and T7 bacteriophage, the particles in the

[4] Investigations on electron microscopy were carried out in the Department of Analytic Chemistry, University of Illinois, under direction of Prof. G. L. Clark.

X. pruni phage filtrate seemed to have a low electron density, suggesting that they may be flat or that they may contain some water of hydration when in suspension and flatten upon drying.

Particles observed in the phage suspension were not always of uniform size. This may have been due to the possibility (a) that the larger particles were aggregates rather than individual bacteriophage units; (b) that the particles had occluded bacterial fragments; or (c) that the phage suspension was a mixture of several types of particles, such as the suspensions used by the earliest electron microscope workers in observing the attack of bacteriophages on *Escherichia coli* (2). By analogy with the electron micrographs of other bacteriophages, the spherical particles approxi-

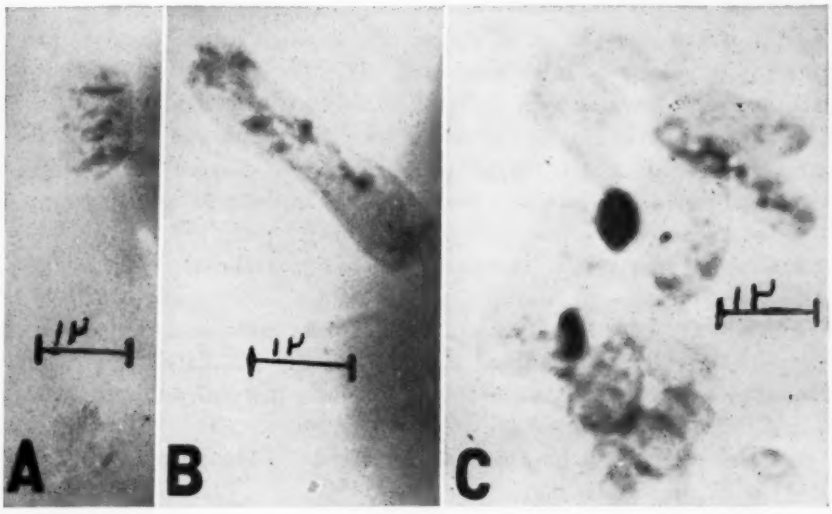

FIG. 2. Electron micrographs of lysed cells showing the region where rupture apparently occurred and some dense areas in the bacterial shell. (A) single cell, (B) double cell, (C) clump of cells.

mately 50 mμ in diameter and those with "tails" are assumed to be the bacteriophage. Evidence is lacking that these spherical particles or their "tails" are the actual infectious units.

Bacterial cells remaining after lysis (Fig. 2) show the place where rupture apparently occurred and some dense areas of the bacterial shell which may be adsorbed particles. Phage placed on established bacterial growth on agar slants lysed the area of the growth where streaked.

DISCUSSION

The aging of *Xanthomonas pruni* bacteriophage in suspension (lysed culture filtrate) for 20 years exceeds the 12-year aging period reported for *Bacterium stewarti* bacteriophage in sealed tubes at room temperature (9). However, this survival period of a bacterial virus is surpassed by the reported 52-year survival of a plant virus (tobacco mosaic virus), in dry tobacco-leaf tissues stored at room temperature (4).

The specificity of the *Xanthomonas pruni* bacteriophage offers a convenient phage-lysis technique for the identification of *X. pruni*. With other bacteriophages the technique has been successfully demonstrated for plant pathogens, *Erwinia amylovora* and two strains of *Aplanobacter stewarti* (7). Since *X. pruni* phage is stable at room temperature and apparently specific in its lysis, the phage-lysis technique is of considerable utility in the general routine identification of the bacterium and the diagnosis of plant diseases caused by this organism. Once the phage is prepared for storage the technique is simple and does not require expensive or complicated equipment or any special skill. For such use samples of 10^{-9} titer bacteriophage have been stored.

The size of the particles assumed to be *Xanthomonas pruni* bacteriophage is of the same order of magnitude as some other phages when determined by electron microscopy (2, 5). By ultrafiltration, however, the *X. pruni* phage has been reported to be 11 mμ in diameter (10). This estimated size was calculated from the smallest membrane pores (33.84 mμ diameter) through which the phage passed. The filtrate secured through these membranes did not cause lysis upon its initial addition to young cultures but required a subsequent transfer in a young culture before lysis occurred. At that time this was considered to mean that the phage was not in sufficient concentration in these filtrates to bring about visible lysis when originally added to a young culture. Lysis from original inoculum occurred with filtrates obtained through membranes with pore diameters as small as 68.32 mμ (10). If this is taken as the end point in filtration, the calculated size of the phage would be 53 mμ which agrees with the size of particles recognized by electron microscopy. The 11-mμ size approximates the smaller dimension of the phage "tails." The results from ultrafiltration and electron microscopy suggest (a) that the appendage is removable from the particle, (b) that the ultrafiltration through 33.84-mμ pores permitted only the appendages to pass, and (c) that these appendages after a period of association with the proliferating host cells then become the complete and specific bacteriophage. The suggestion that the appendage may contain the specificity portion of bacteriophage is further supported by the fact (a) that phages or their precursors require an association with a given organism through a series of transfers in order to become demonstrable (6, 8, 9), (b) that some particles assumed to be *X. pruni* bacteriophage in the electron micrographs, are without appendages, and (c) that specificity of a phage is related to the host on which it is propagated from its common origin (6, 8, 9). Furthermore, the orientation of adsorbed particles on bacterial cells appear to be with appendage toward the cell as is also suggested by some of the adsorbed particles in the electron micrographs of *Escherichia coli* bacteriophage (5) when enlargement prints are examined. However, drawn illustrations show the adsorbed orientation with the appendage away from the cell (3).

SUMMARY

Xanthomonas pruni bacteriophage remained viable for 20 years in sealed tubes at room temperature in darkness.

The bacteriophage titer was increased to 10^{-9}.

The bacteriophage is specific for *Xanthomonas pruni*.

The particles assumed to be bacteriophage are about 50 mμ in diameter.

Some particles appear to have an appendage about 10 by 100 mμ and resemble the T3 and T7 *Escherichia coli* bacteriophage particles.

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REPORT AND ABSTRACTS OF THE THIRTIETH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 30th annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California, Berkeley, California, June 22–25, in conjunction with the meeting of the Pacific Division of the A.A.A.S. Approximately 100 persons attended the meetings. Arizona, British Columbia, California, Hawaii, Oregon, Utah, and Washington were represented. Thirty-five papers were presented on June 22 and 23. A field trip to the east and south of the Bay region on June 24 provided a survey of a wide variety of diseases. Four discussion sessions were held Friday morning, June 25. Topics were: "Control Measures for Plant Viruses," "Problems in the Identification and Classification of Fungi," "Recent Developments in Chemical Control of Plant Diseases," and "The Role of Plant Breeding in the Control of Plant Diseases." Presentation of six papers in a joint session with the American Society of Plant Physiologists and Botanical Society of America on "Biology of Fungi" concluded the program on Friday afternoon.

At the business session June 23 the following officers were elected for the calendar year 1949:

President: George W. Fischer
Vice-president: Wm. C. Snyder
Secretary-Treasurer: George A. Zentmyer (2 year term)
Councilor: L. C. Cochran (2 year term)

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Effects of amino-acridine derivatives on tissues of potato (Solanum tuberosum). ALPEN, E., JEAN DUFRENOY, and W. D. KUMLER. Discs punched out of the tubers of various genotypes of *Solanum tuberosum* were immersed in a shallow layer of solutions of amino acridine derivatives of concentrations ranging from 0.0004 M to 0.001 M. The relative "cytotoxicity" of the various compounds was evaluated in terms of the number of days or weeks during which the discs remained whole and sound, of the rate of formation of cicatrization layer at the cut surface, of the proportion of cells retaining the property of actively adsorbing vital dyes in their vacuolar solution, and last, of the rate of amylolysis and the fate of amyloplasts and mitochondria.

Susceptibility of some citrus species and other plants to the citrus-root nematode, Tylenchulus semipenetrans. BAINES, R. C., O. F. CLARKE, and W. P. BITTERS. In the field under conditions of general natural infestation of the citrus-root nematode (*Tylenchulus semipenetrans*) roots of the following were found to be infested moderately to severely: *Citrus aurantium* (29 varieties), *C. aurantifolia, C. celebica* var. *southwickii, C. grandis* (8 varieties), *C. hystrix, C. ichangensis, C. limon* (10 varieties), C. *medica,* C. *paradisi* (5 varieties), C. *reticulata* (6 varieties), C. *sinensis* (18 varieties), *C. tiawanica, Atalantia citroides, Fortunella* sp., *Microcitrus australasica, Microcitrus australasica* var. *sanguinea;* the following hybrids—*C. aurantifolia* × *F.* sp., *C. ichangensis* × *C. grandis, C. limon* × *C. paradisi, C. macroptera* × *C. reticulata, C. reticulata* × *C. grandis, C. reticulata* × *C. sinensis, C. sinensis* × *Poncirus trifoliata, C. tachibana* × C. *reticulata,* Citrange × C. *sinensis,* P. *trifoliata* × C. *limon,* P. *trifoliata* × C. *paradisi, P. trifoliata* × *C. sinensis* × *F.* sp. Roots of the following were free from this nematode: *Balsamocitrus dawei, Clausena lansium, Murraya paniculata* and *Severinia buxifolia.* Three of 20 *P. trifoliata* also were free from the citrus-root nematode. The resistant plants may be valuable in developing nematode-resistant rootstocks for citrus.

A leaf and twig blight of California laurel, Umbellularia Californica Nutt. BARRETT, J. T. A leaf and twig blight of the California laurel has been observed on the Berkeley campus for the past fifteen years. Many trees are slightly infected, others severely, and a few have been removed because of the disease. Infection by the pathogen, a species of *Macrophoma,* takes place mainly in the leaf tips from water drops carrying spores, and through the stomata which occur only on the dorsal side. From the leaf the fungus enters the twig which soon dies. If on a branch or limb, a canker may result and continue to develop two or more years. Tests have shown that infection may result within 48 hours after spores are placed on uninjured leaves and young shoots. Inoculation of young shoots of genera related to the laurel, such as *Persea gratissima, Persea india, Persea lingue, Cryptocarya mierri,* and camphor showed all to

be mildly susceptible. A number of unrelated forms subject to attack of Macrophomas were resistant to the laurel pathogen. All attempts to find in nature or to induce in culture a perfect stage have, to date, failed.

Some conditions contributing to the development and germination of oospores in the genus Phytophthora. BARRETT, J. T. In a previous report (Phytopath. 38: 2) the production of oospores by pairing species and strains of *Phytophthora* was reported by the writer. Similar results have been attained by pairing male and female strains of *Phytophthora drechsleri* with several isolants of *P. palmivora*, which demonstrate sexual compatibility between these two species and confirm some results of previous workers that two sex strains occur in the latter species. Oospores resulting from crossing the two sex strains of *P. drechsleri* have geen germinated, and 22 cultures from as many oospores have been established. No oospores have been found in any of these single cultures but when backcrossed with the two parents, 14 reacted with the male parent only, and six with the female only. In each case an abundance of oospores resulted. Oospores resulting from crossing two isolants of *P. infestans*, one from potato and one from tomato, with the female *P. infestans*, were also germinated, but only one culture from them has as yet been established. When backcrossed with the female *drechsleri* parent this culture produced an abundance of oospores, but none when mixed with the male strain.

Soil disinfestation in citrus orchards against armillaria root rot. BLISS, DONALD E. *Armillaria mellea* was found to a depth of 9 feet in sour-orange roots. Treatments with carbon disulfide were made in nearly undisturbed orchard soils containing artificially inoculated citrus root segments, 15 to 57 mm. diam. and 17 cm. long, planted at 1-foot intervals in vertical series 8 or 10 feet deep. Culture tests were made after 75 or 98 days. Charges of CS_2, applied at 18-inch, staggered intervals, killed armillaria in roots as follows: In coarse sandy loam near Azusa, California, 59 ml. at 1 foot killed from 1 to 7 feet, inclusive; 118 ml. at 1 foot killed from 1 to 8 feet; and 118 ml. at 6 feet killed from 3 to 10 feet. Near Rivera, in fine sandy loam underlaid with a clay-bearing substratum (5 to 6 feet) and sand, 44 ml. at 1 foot killed from 1 to 3 feet; and both 59 and 88 ml. at 1 foot killed from 1 to 5 feet. Surface charges failed to penetrate the clay substratum, deep charges being required. *Armillaria* remained viable in nontreated roots. *Trichoderma viride* developed in 54 to 100 per cent of cultures from treated roots, but in only 25 per cent from nontreated roots.

Exosporina branch blight of grapefruit in southern California. CALAVAN, E. C., and J. M. WALLACE. A branch blight of Marsh grapefruit was observed in November, 1945, in the Coachella and Imperial valleys of California. The withered branches contained spores of a toruloid fungus beneath the loosened periderm and frequently were associated with an anomalous form of trunk and branch gumming, with psorosis, or with both. Although confined principally to the smaller branches, blight lesions occasionally extended down primary branches to the trunk. The disease appeared to be more severe on weak trees than on healthy ones. Isolates of the fungus appeared nearly identical to isolates of *Exosporina fawcetti* Wilson from walnut. Ninety wound inoculations with pure cultures of *E. fawcetti* (from grapefruit) in April and July, 1947, on Marsh grapefruit trunks, branches, and twigs produced lesions in all cases. Forty-eight check wounds healed rapidly without infection. The fungus was easily recoverable from these inoculations as late as May, 1948.

Differences in susceptibility of lemon strains to dry bark. CALAVAN, E. C., and F. A. WHITE. Apparent differences in susceptibility of certain lemon strains to dry bark were observed in a few orchards in 1946, but the determination of resistant and susceptibile selections has been retarded by the general lack of detailed source records for most diseased plantings. Recent surveys in cooperation with Citrus Field Research, Inc., of Santa Barbara County, California, involving 15 nursery strains for a total of 3,917 trees in 8 orchards where careful records had been kept, revealed marked differences in the amounts of dry bark present in various strains. Six of the 15 strains were entirely free from dry bark, whereas the other 9 strains broke down in percentages ranging from 7 to 73 per cent. The 9 susceptible strains developed dry bark in all orchards where they were known to be planted. Tree susceptibility in the orchards studied (age 9 to 16 years) showed no important relationship to age, variety (Eureka and Lisbon), or rootstock.

Effects of various chemicals as growth-regulators. CARLO, P., JEAN DUFRENOY, and PETER SAH. Various compounds endowed with marked fungistatic activity and notably

Vitamin K_5 synthesized at the College of Pharmacy, U. C. Medical Center, San Francisco, have been demonstrated to show properties of growth-regulating hormones at concentrations ranging from 1 to 100 p.p.m. Their effect on the rooting of cuttings or on the germination of seeds was also studied in the presence of dithobiuret, which, by itself has remarkable growth-regulating properties.

Mutation in bean rust uredospores in cold storage. DUNDAS, B. Uredospores of a number of forms of bean rust produced on detached bean leaves in Petri dishes collected during the summer of 1946 were subject to varying temperatures before being placed in storage at 0° F. in the fall of that year. When taken out in July, 1947, very few of the spores were viable, some collections giving no infection and the rest only a few pustules. Isolations from these pustules, tested on nine differential varieties, were in every case different from the form as stored, and in most cases different from previously described forms. Numerous isolates from a mixture of uredospores of 15 pure forms yielded only eight forms, six of which were different from any previously described. Since the unmixed uredospore collection of the other two forms yielded only mutant forms it is considered likely that these also may have been mutants, especially since both of these forms have come out as mutants from other pure forms. In all, nine mutant forms have been isolated. Mutation to the same form has occurred in several different forms and two or more mutants have been obtained from a single pure form. No consistent increase or decrease in virulence has been observed.

Disinfectant washes for the control of decay in apples and pears. ENGLISH, HARLEY. Various disinfectant washes were tested for the control of blue mold (*Penicillium expansum*) and bull's-eye rot (*Gloeosporium perennans*) of apples and gray mold rot (*Botrytis cinerea*) of pears, using punctured, inoculated fruit. Of the phenol derivatives tested, sodium chlororthophenylphenate proved most effective whether used in a rinse or in an alkaline wash, reducing both blue and gray mold rots by 80 to 95 per cent. The thiocarbamates were ineffective against blue mold, but the soluble salts were highly effective against gray mold. Neither the phenol derivatives nor the thiocarbamates controlled bull's-eye rot. The quaternary ammonium compounds showed promise only in the control of bull's-eye rot. Alkyl dimethyl benzyl ammonium chloride was effective against the latter whether used in a final rinse or in an acid wash. With the exception of several mercury compounds, none of the other fungicides tested was so effective as sodium chlororthophenylphenate for the control of blue and gray mold rots. In semi-commercial tests with apples, sodium chlororthophenylphenate in a final rinse reduced decay 43 to 75 per cent. In similar tests with pears, the addition of this compound either to a final rinse or to the alkaline wash reduced decay 65 to 86 per cent.

The effect of particle size and solubility of sulfur in carbon disulfide upon its toxicity to fungi. FEICHTMEIR, E. F. The influence of allotropic form, particle size, and type of grinding upon toxicity of sulfur toward fungi has been investigated using particle size ranges of $\pm 1\,\mu$. These narrow size ranges were separated by means of liquid sedimentation. The results are itemized. 1. With sulfur particles 1–2 μ in diameter the concentrations of CS_2-insoluble and CS_2-soluble sulfur, respectively, in μg per ml. for 50 per cent reduction in germination or infection were as follows: for germination of conidia of *Sclerotinia fructicola*—3.9 and 13, for germination of conidia of *Erysiphe graminis hordeii*—0.6 and 2.1, for protection against *Uromyces appendiculatus*—30 and 80, and for protection against *E. polygoni*—1.9 and 12. 2. The LD 50 against conidia of *S. fructicola* for CS_2-soluble sulfur particles of 1μ, 3μ, 5μ, and 8μ radius decreased directly as the surface area per unit weight increased. 3. Agglomeration reduced fungicidal effectiveness. 4. Large particles remained toxic longer than small ones. 5. Micronized sulfur showed no greater innate toxicity than Raymond mill ground sulfur. In addition, an amorphous form (particles approximately 2μ in diameter) produced by evaporating an acetone solution of sulfur gave a high toxicity against conidia of *S. fructicola*—LD 50 of 0.13 μg per sq. cm.

Curly top of muskmelon. GIDDINGS, N. J.

A canker disease of figs. HANSEN, H. N. A canker disease of fig trees has been under investigation since late summer of 1947 when it was first observed in one of the highest producing orchards in California. The disease so far has been seen only on the variety White Adriatic and it appears to be confined to the older parts of trees since lesions have not been found on wood less than four years old. The younger cankers are rather inconspicuous and usually consist of a slight cracking of the outer bark. When scraping away the bark down to the cambium, one may find that a half-inch

crack on the surface often accompanies a six-inch canker at the cambium. Isolations from the margins of cankers consistently yielded a bacterial organism which when inoculated into the cortex of six-year-old wood of a fig tree gave rise to typical cankers. A similar disease occurs on fig trees in Italy where the causal organism is known as *Bacterium fici.*

Compatibility and sex in Hypomyces solani f. cucurbitae. HANSEN, H. N., and WILLIAM C. SNYDER. This heterothallic fungus occurs in four sex forms: hermaphrodites, females, males, and neuters. The compatibility group to which individuals of the above groups belong is determined by applying conidial suspensions of each of them to two tester strains, one known to be + and the other –. If the individual produces perithecia when mated with + it belongs in the – compatibility group and *vice versa.* The neuters, which are sexless and therefore unable to produce perithecia when mated with the + and – tester strains nevertheless give a positive reaction in that a conidial suspension of a neuter will completely inhibit trichogyne formation in one of them and not in the other. Neuter + will inhibit trichogyne formation in tester – and *vice versa.* Thus the factors for heterothallism + and – are also present in sexless fungi.

Methods of inoculation of barley with the stripe disease, Helminthosporium gramineum. HOUSTON, BYRON R., and JOHN W. OSWALD. Numerous tests were conducted to determine a practical yet efficient method of inoculation of barley with the stripe disease fungus, *Helminthosporium gramineum.* Tests included spore suspensions applied to seed with and without vacuum, dried mycelial masses applied to the seed, and germination of seed in contact with growing cultures. Only the latter method resulted in consistently high percentages of infections. This method consisted of placing dry barley seed on the surface of a four-day Petri dish agar culture, inverting a similar culture over the seed, and allowing the seed to germinate. Employing this method, twelve time intervals of inoculation (constant 12° C.) ranging from 24 to 120 hours showed at 24 hours, 53 per cent infection; at 48 hours, 86; at 60 hours, 94; and all over 60 hours a percentage infection over 94. Seed dried for 10 days following inoculation resulted in no significant difference in percentage of disease except for the 24-hour interval. Inoculations carried to the same degree of seed germination at six temperatures ranging from 6° to 30° C. resulted in a high percentage of disease at all temperatures. Planting date and prevailing temperatures had little effect upon percentage of disease. Best infection occurred with this method at 12° C. for 96 hours.

A disease index for verticillium wilt of pepper. KENDRICK, J. B. JR., and JOHN T. MIDDLETON. Seedlings of *Capsicum frutescens* L. infected with *Verticillium albo-atrum* Reinke and Berth, have a characteristic stunting which may be classified and analyzed statistically. Stunting of inoculated plants is caused by reduced growth of internodes not fully expanded at the time of infection. A disease index is derived by comparing the individual length of an infected plant with the mean length of noninfected plants. Measurements are taken at the time of inoculation and four weeks later, for both inoculated and noninoculated plants. The difference represents the interim growth for four weeks of infection and normal expansion. The quotient computed by dividing the mean four-week growth of noninoculated plants by the same measurement of inoculated individual plants is the disease index. Relative severity is thus evident by the index, the greater the index the more severe the disease expression. This disease index is useful in a disease resistance breeding program where it is essential to preserve plants for subsequent hybridization and seed production. Confidence limits are established statistically for populations of noninoculated plants. Inoculated plants with an index within the confidence limits are regarded as highly resistant. Arbitrary classes may be established to include plants with different degrees of disease resistance.

Observations on the effect of 2,4-D on citrus fruit-stem dieback. KLOTZ, L. J., and W. S. STEWART. Dying of fruit stems occurs generally throughout California citrus orchards and causes important losses of crop in certain sections. Dying starts near the fruit button and extends a few inches to as much as 3 feet up the stem. The fruit then wilts or drops. Cause of the trouble is attributed to adverse climatic and soil factors acting to modify the physiology of the tree. During periods of water stress, gums may form and interfere with subsequent transport of water. The same stimuli may incite maturation of the abscission layer. Affected stems are invaded by fungi and bacteria which play an important secondary role in killing the stems after the initial injury. The chemical, 2,4-dichlorophenoxyacetic acid, applied in May and June, has been found to delay abscission and fruit drop, and to reduce fruit-stem dieback as much as 80 to 90 per cent.

Control of citrus brown rot, gummosis, and rind oil spotting by long exposures to low, moist heat. KLOTZ, L. J., and H. D. EVANS. The development of phytophthora brown rot in inoculated lemons, incubated 12 to 72 hours at 45°, 55° or 65° F., was stopped by an 18-hour exposure to air at 105° F., and 70 per cent to 96 per cent relative humidity. At 65° F. the fungus produced brown rot spots $\frac{1}{16}$ inch in diameter after 48 hours, and $\frac{1}{2}$ inch spots after 72 hours incubation, making the fruit unfit for use even though the heat treatment checked further development of the decay. All the other lemons used in the experiments had no blemishes. The treated fruit following 4 minutes' immersion in soda ash (1¾ per cent)–soap (¼ per cent) solution at 120° F. or water at 120° F. did not develop rind oil spots. Budded lemon trees on sweet stock can tolerate a wet bulb temperature of 98° F. for 25 hours, an exposure lethal to the gummosis fungi.

Effects of combining fungicidal treatments and coating of seeds. LEACH, L. D. Tests were conducted in both pasteurized and Pythium infested soil to determine the effects of coating and incorporation of fungicides on emergence and infection of seedlings. In most cases, the coating of tomato seeds resulted in a slight delay of emergence, but did not reduce the number of seedlings. Coating alone reduced pre- and post-emergence infection, but the addition of fungicides such as Arasan (tetramethyl thiuram disulfide) and Phygon (2,3-dichloro-1,4-naphthoquinone) increased protection. The delay of emergence following coating of tomato seed previously treated with $HgCl_2$ or New Improved Ceresan (ethyl mercury phosphate) dip, was greater than the additive delays from coating and treating separately. The inclusion of organic mercury compounds in the coating, resulted in reduced and retarded emergence. With processed sugar beet seed a neutral reaction of the coating was most favorable for emergence, but an acid reaction enhanced protection. Coating alone provided about half as much protection against Pythium infection as Arasan or Phygon in the coating material. Coating onion seed with or without Arasan at a dosage of 5 per cent of the seed weight, neither reduced nor delayed emergence. However, emergence was delayed by 50 per cent when Arasan at 75 per cent of the seed weight, was included in the coating for smut control.

Attraction of the vascular bundles for dodder haustoria in healthy and curly-top infected beet petioles. LACKEY, CHARLES F. Comparisons are made between dodder haustoria and beet leafhopper stylets in their ability to go directly to the vascular bundles of the beet petiole. Both go directly to the bundles in the majority of cases and can turn to a bundle when continuation in the original direction would have passed it. Neither dodder nor leafhopper will penetrate very deeply on the upper side of the petiole in tissue well away from the vascular bundles. Neither one will live long without vascular contact. Bennett (Jour. Agr. Res. 48: 665–701) showed that in 46 per cent of the cases the leafhopper stylets went directly to the bundles, 46 per cent started toward bundles but did not go deeply enough, and only 8 per cent were on the concave side of the petiole in tissue well away from the bundles. In healthy beet petioles the dodder went directly to the bundles in 97 per cent of the cases and missed in only 3 per cent. These data suggest a similar attraction of the vascular bundles for both the leafhoppers and dodder. In curly-top infected petioles only 53 per cent of the haustoria went directly to the bundles and in 47 per cent they missed the bundles or did not go deep enough. No studies regarding the effect of curly top infection on the feeding of the beet leafhopper have been reported.

Studies on the chemotherapy of potato virus diseases. LOCKE, SETH B. Treatment of a hill of leafroll-infected Netted Gem potatoes with 0.2 per cent 2,4-dichlorophenoxyacetic acid resulted in the complete masking of symptoms in the new growth produced after treatment, and also in the first vegetative generation of plants grown from the tubers of the treated plant. Grafts made at intervals from the first vegetative generation progeny of the treated plant to healthy plants showed no transmission at first, and later, increasing proportions of transmission up to 100 per cent. Symptom development was slower in the earlier transmissions and more rapid in the later ones relative to rate of symptom development following transmission from non-treated leafroll infected plants. These results are interpreted as indicating a reduction in amount of active virus in the treated plants and not merely masking of symptoms. Reinoculation of first generation, vegetative progeny of the treated plant by grafting with scions from non-treated leafroll plants failed to induce symptom development. This may indicate that amounts of the active chemical sufficient to inhibit virus multiplication were present in the plants at the time of reinoculation.

Pythium wilt of Phaseolus, Pisum, and Vigna. MIDDLETON, JOHN T., and W. C. SNYDER. Pythium wilt of bean, pea, and cowpea is characterized by a light brown, watersoaked discoloration of the stem, usually ½ to ¼ inch wide, originating at the ground level and progressing upwards 6 to 12 inches, followed shortly by a conspicuous unilateral wilting of the plant. Susceptible hosts are most subject to infection with the advent of flower production. The disease is caused by several species of *Pythium: P. aphanidermatum, P. debaryanum, P. irregulare, P. oligandrum, P. splendens,* and *P. ultimum.* Varieties of *Phaseolus vulgaris* found naturally infected include: Kentucky Wonder, Red Kidney, Small White, Standard Pink, Small White Lima, and Ventura Lima. Varieties of *Pisum sativum* found naturally infected are: Alderman, Little Marvel, and Progress. Several varieties of *Vigna sinensis* have been found naturally infected. In California the disease is encountered wherever susceptible hosts are grown, but perhaps is more commonly noted in the Salinas and lower San Joaquin valleys. Although several *Pythium* spp. are concerned, *P. ultimum* is generally responsible for causing the disease in coastal areas, while both *P. ultimum* and *P. aphanidermatum* cause the malady in the interior.

A late-breaking virus disease of potatoes. MILBRATH, J. A., and W. H. ENGLISH. An unusual graft-transmitted virus disease of potatoes has been found in Oregon. Plants grow normally until late in the season and then they become off-color, the stems stand erect, aerial tubers develop in nearly every leaf aril, and the leaves became yellowed and rolled. Plants affected with acute symptoms remain small, yellowed, and have thickened nodes and aerial tubers. Outstanding points which differentiate this disease are as follows: it is tuber perpetuated for at least three generations; normal-appearing plants from infected tubers develop symptoms late in the season as plants reach full size; tubers are reduced in size, normal in number, and without internal symptoms; tubers from an infected hill may produce normal plants, weak plants, and plants with severe or mild symptoms; a single tuber cut in several pieces may produce plants which also show this variation in symptom development. This combination of symptom expressions does not fit any other virus disease associated with potatoes.

Arsenite injury to grape canes through leaf scars. NELSON, K. E., and WM. B. HEWITT. Sodium arsenite applied as a dormant spray to the canes, arms, and trunks of Thompson seedless vines (*Vitis vinifera* L.), on dry soil plots, caused appreciable injury as evidenced by bud killing and sharply defined necrotic areas in the xylem tissue below the leaf scar. Vines in a wet soil plot had only minor xylem injury with little bud killing. Vines sprayed only on the arms and trunks had considerably less injury, it being confined chiefly to the buds at the bases of the canes. Histological study showed that the leaf scar periderm was formed before leaf fall. Most of the vessels of the leaf traces in scars collected in December after leaf fall were open with no covering over the broken ends and little to no wound gum within the vessels. Some of the leaf scar traces collected in January were partially plugged and the remainder entirely plugged with gum. By February, plugging of the leaf scar traces was complete. A study of damaged buds indicated the sodium arsenite penetrated to the xylem tissue through the vessels of the leaf trace. From there, necrosis extended downward as far as four inches below the leaf scar and also upward into the bud.

The influence of fungicidal dusts upon the rooting of cypress and tamarix cuttings. NEWTON, WM. In the propagation of Cypress and Tamarix by cuttings, the loss of cuttings is greatly diminished by the simple practice of dipping the bases of the cuttings into dry Fermate (ferric dimethyl dithiocarbamate). This practice is more effective in the promoting of rooting and survival than a one-hour immersion in a solution of naphthalene acetic acid which contains 10 p.p.m. The inclusion of a trace of naphthalene acetic acid with Fermate and other fungicides had little significant effect upon the cuttings.

Leaf roll, net necrosis, and stem end browning in Netted Gem potatoes in California. OSWALD, JOHN W., and JAMES B. KENDRICK. Leaf roll is very serious in Netted Gem potatoes because current season infection markedly reduces yield and causes net necrosis in the tubers. Reduction in yield is directly proportional to the severity of plant symptoms. In addition to net necrosis, Netted Gem potatoes develop stem end browning. Tuber indexing showed net necrosis was definitely associated with leaf roll, whereas, stem end browning was not. Tubers from 90-day-old plants had only a trace of net necrosis, but after six weeks' cellar storage (about 45° F.) and cold storage at 36° F., showed 23 and 5 per cent respectively. Mature tubers harvested after killing frost, showed 40–50 per cent net necrosis and 10–15 per cent stem end browning with no subsequent increase

in either disease in either cellar or cold storage. Tubers from 90-day-old plants showed no stem end browning and subsequently developed very little in either type of storage, indicating the conditions bringing this about occurred between early and normal harvest. Rolling, removing, and killing plants before and after frost had little effect on the development of either type of browning. Stem end browning is definitely more severe on muck soils (39.3 per cent) than on sandy soils (4.6 per cent).

Sieve-tube necrosis, an early symptom of collapsing and declining lemon trees. SCHNEIDER, HENRY. Forty-two apparently healthy 14-year-old lemon trees budded on grapefruit stock exhibited necrosis of part or all of the sieve tubes just above the bud union in May, 1947. At ¼ inch and 6 inches above the union, necrosis was less extensive or absent, and none was observed below the union. The phloem of scaffold branches and twigs was usually normal. Starch, which was abundant in the wood of the roots of most of the trees in May, 1947, subsequently disappeared in most of them. By May, 1948, many of the trees exhibited yellowing of the leaves and partial defoliation; the fruit ripened prematurely and was of small size; and the more severely affected trees had failed to make a growth flush. The results indicate that necrosis of sieve tubes is an early symptom which causes starvation of the roots, which usually causes top symptoms similar to those of lemon decline, and occasionally causes a collapse of trees. In six other orchards on grapefruit stock and in one on sweet orange stock, observed over a shorter time, similar symptoms have been observed; but in two of the orchards only a few trees were affected. New phloem was present in trees that had collapsed or declined and then recovered.

Inheritance of spotted-wilt resistance in tomato. SMITH, PAUL G., and M. W. GARDNER. A high level of resistance to spotted-wilt in tomato has been demonstrated in the Red Currant tomato (*Lycopersicon pimpinellifolium*) under conditions of field infection, but it is susceptible when artificially inoculated. German Sugar, a variety of *L. esculentum*, is moderately resistant to field infection. Hybrids with Red Currant and the cultivated tomato have yielded lines with an intermediate level of resistance. These remain comparatively free of spotted-wilt under moderate epidemic conditions, but are badly diseased under severe epidemic conditions. The Red Currant level of resistance has not been recovered in any of its progeny, and no evidence of simple Mendelian inheritance of resistance has been observed. Hybrids of German Sugar and the commercial varieties yield F_1 and F_2 populations intermediate in resistance. In back crosses of the F_1 to the resistant parent, the German Sugar level of resistance was not recovered. The back cross of the F_1 to the susceptible parent yielded a level of resistance intermediate between them. Again no evidence of Mendelian segregation was observed. The reaction of the resistant and partially resistant varieties varies markedly in different years and localities, the causes of which are not clear.

Diseases of Guar (Cyamopsis psoraloides). STREETS, R. B. Guar has been grown in the Southwest in recent years as a summer green-manure crop and for the monogalactan gum of the seeds. No severe losses from plant diseases have occurred in Arizona or elsewhere, but 14 diseases and injuries, some more conspicuous than injurious, have been recorded. The branched Mesa variety is highly resistant to two diseases, root-knot caused by *Heterodera marioni*, and root rot caused by *Phymatotrichum omnivorum*. The unbranched, early-maturing Texsel variety is moderately susceptible to root rot, but resistant to Sclerotium rot. Some losses occur from Fusarium and Rhizoctonia root rots, and *Sclerotium rolfsii* is injurious in wet soils. Mosaic caused losses of seed yield up to 50 per cent in 1943 but has not reappeared. Alternaria leaf spot is the only common foliage disease and may cause more or less defoliation in years of greater than normal rainfall and humidity. Many plants of unbranched varieties are stunted and finally die from an injury, of unknown cause, to the growing point when the plants are 6 to 18 inches high. Two diseases reported elsewhere have not been observed in Arizona: lethal virus (Oklahoma) and powdery mildew (India). Five minor diseases not mentioned above have been observed.

Profitable use of root-rot-infested irrigated land. STREETS, R. B. Cost analyses have been made on seven rotations with cotton or flax as the principal cash crop in comparison with continuous cotton on root-rot-infested irrigated land for the years 1944–47, inclusive. To the normal cost of growing the crops was added the expense for materials and labor used in different treatments and rotations. The average per acre profits for the replicated plots for 1946 and 1947 were computed, as the effects of treatment and rotation are cumulative. Root rot, caused by *Phymatotrichum omnivorum*, was reduced in varying amounts, but not eliminated from the plots, but the net returns per acre were increased. The rotations in ranking order of profit (1946–47 averaged)

were as follows: (1) barley, guar for seed, sulphur and nitrogen, cotton (two-year rotation); (2) winter legume for green manure, cotton; (3) manure in furrows under cotton rows; (4) early guar for seed, flax; (5) guar for green manure, flax; (6) manure in furrows plus sulphur and nitrogen; (7) sesbania for green manure, flax; and (8) continuous cotton. Rotations 3 and 6 require too much labor and expense for materials to be useful in general practice.

The effect of temperature on the taxonomic characters of Verticillium albo-atrum Rke. et Bert. WILHELM, STEPHEN. A temperature difference of 3°–6° C. within the growing range (10°–31° C.) of *Verticillium albo-atrum* Rke. et Bert. may produce in this fungus marked differences in cultural appearance and in morphological characters, particularly of the resting stages. Colonies grow at low temperatures (10°–22° C.) or during the winter at Berkeley, California, are jet black and growth consists almost entirely of thick microsclerotial crusts. At higher temperatures (25°–31° C.) or during the summer at Berkeley, colonies are creamy white and have only sparse development of microsclerotia. Thus the resting structures of this fungus, in particular the microsclerotia, and colony appearance are not reliable characters upon which to separate species. Colonies grown at low temperatures answer to the concept of *V. Dahliae* Kleb. as interpreted by Van der Meer and Keyworth and to the *Niger* group of *V. Dahliae* as described by Berkeley. Only the wild type or conidial constituent of *V. albo-atrum* respond as described to temperature, the stable white mycelial variants being unaffected.

A dwarfing virus disease of bramble fruits. WILHELM, STEPHEN, H. E. THOMAS, and D. D. JENSEN. A graft transmissible disease of bramble fruits, serious in California mainly in the Logan variety, is proven to be of virus nature. Diseased Logans are characterized by marked dwarfing of cane growth, weak development of fruit laterals, downward cupping and yellow-bronze coloring of the leaves, premature reddening in the fall, and precocious development of basal buds giving plants, late in the season, a bunchy appearance. Plants are slow to start in the spring, and when diseased a year or more are unproductive. The varieties and species of blackberry related genetically to the Logan, i.e., Mammoth, Cory's Thornless, and Phenomenal, and the Pacific Coast trailing blackberry (*Rubus ursinus* Cham. & Schlecht.) are readily graft inoculated and all but the last affected severely. Less severe symptoms are produced in the varieties Boysen, Young, and Nectar. In these varieties the disease has not been identified in the field. Preliminary tests with raspberry indicate that it may act as a symptomless carrier. The disease was produced in Logan when inarched to the wild Pacific Coast trailing blackberry in 5 out of 35 trials, indicating that this wild blackberry harbors the virus.

Relation of temperature and moisture to the effectiveness of sodium pentachlorophenate as an eradicant spray against Sclerotinia laxa. WILSON, E. E. Measurable eradicative action by a sodium pentachlorophenate spray preparation used against sporodochium-borne conidia of *Sclerotinia laxa* on almond twigs, was not detected during the short period required for the spray to dry on the twig, or during dry weather. Continuous wetting of the twigs was more favorable to eradicative action than intermittant wetting; the percentage mortality of conidia on sprayed twigs increased progressively as the period of moisture continued for eight days. Heavy rains not only checked destruction of conidia by the spray, but allowed new sporodochia to develop. Mortality of sporodochium-borne conidia receiving the sodium pentachlorophenate spray rose with successive increases of temperature between 7° and 30° C. Apparently this rise in mortality was occasioned by an increased eradicative action of the spray and not by high temperature since exposure of conidia to 24° and 30° C. did not materially affect their subsequent germinability. In the field such high temperatures did not occur, the range being from about 5° to 13° C. Within this range, development of sporodochia and the consequent production of conidia on untreated twigs increased rapidly with increased temperature. In a similar manner temperature affected the development of sporodochia on treated twigs that had been exposed to a considerable amount of rain after treatment with sodium pentachlorophenate.

Apricot jacket rot. YARWOOD, C. E. This disease, also called green rot, calyx rot, and blossom rot, is caused by *Sclerotinia sclerotiorum, Botrytis cinerea, Monilinia laxa,* and *Monilinia fructicola,* the fungi probably being of importance in this order. Infection starts in the senescent calyx, but may invade the enclosed or contiguous young fruit and cause it to rot and then drop. One or the other of the first two fungi have been regularly present in normal almond and apricot blossoms in all localities explored from 1943 to 1948 but have caused loss only during prolonged periods of wet weather. Fruit rot by the three genera can be distinguished by the color of the rot (being darkest with *Monilinia* and palest with *Sclerotinia*) and by the characteristic aerial mycelium or sporo-

phores produced after incubating specimens in a moist chamber. In Berkeley the *Botrytis* has predominated, and jacket rot caused by *Sclerotinia* has not been found with certainty, while in the Winters and Hollister areas the *Sclerotinia* has predominated. Jacket rot caused by *Sclerotinia* or *Botrytis* and the twig phase of brown rot caused by *Monilinia laxa* have been controlled by an application of 0.2 per cent Fermate (ferric dimethyl dithiocarbamate) plus spreader at full bloom.

Germ tube growth of some obligate parasites on agar media. YARWOOD, C. E. On washed non-nutrient agar at 22° C. the sporangia of *Peronospora destructor* produced unbranched germ tubes about 500μ long and then ceased growth. With the addition of 2 per cent sucrose, 0.05 per cent glycine, 0.05 per cent calcium glycerophosphate, 0.02 per cent dipotassium phosphate, 0.01 per cent histidine, 0.004 per cent glutathione, and 0.001 per cent disodium alpha tocopherol phosphate to the agar, branched germ tubes averaging about 2600μ with a maximum of 7000μ have resulted in 4 days. Differences in growth on controls and on test media have been greatest at 25° C., though the optimum for the organism is about 16° C. On non-nutrient agar at 16° C., germ tubes of the conidia of *Erysiphe graminis* from barley grew to about 44μ in 2 days, while on agar to which sucrose, Tween 60 (a polyoxyalkaline derivative of sorbitan monostearate), and disodium alpha tocopherol phosphate had been added the germ tubes averaged about 280μ with a maximum of 650μ. On non-nutrient agar at 20° C. the germ tubes of thinly-seeded uredospores of *Uromyces phaseoli* from bean averaged 390μ, while on agar to which sucrose, potassium permanganate, disodium alpha tocopherol phosphate, and glutamic acid had been added the germ tubes averaged about 1000μ.

Western X-disease of Montmorency cherry and its relation to buckskin of sweet cherry. ZELLER, S. M., and J. A. MILBRATH. Montmorency cherry trees infected with "little cherry" bear small green fruits that ripen late. The disease transmitted to sweet cherry causes buckskin disease and transmitted to peach trees causes Western X-disease. A table of 54 cross transmissions of Western X-disease of peach, red-leafed chokecherry disease, and the "little cherry" found in Montmorency cherry and several sweet cherry varieties in Wasco county, Oregon, indicates that all of these diseases are caused by the same virus, or that the infected trees contain the Western X-disease virus. The symptoms of this disease of sweet cherry look the same as those of the buckskin disease of sweet cherry in Green Valley, California. On the other hand, the disease in Oregon has not been demonstrated to react to Mazzard and Mahaleb rootstocks as reported by Rawlins and Parker for the buckskin disease of cherry in California.

PHYTOPATHOLOGICAL NOTES

Zinc Dimethyldithiocarbamate (Zerlate or Karbam White), A Promising Fungicide for Pecan Scab Control.—Home-made Bordeaux mixture, even after more than 30 years of investigations, has been the cheapest and most satisfactory fungicide for the control of pecan scab (*Cladosporium effusum* (Wint.) Demaree), a disease of major importance in the pecan industry. In 1946, tests[1] made on the Moore variety indicated that 3 lb. Copper A or Fermate per 100 gal. would control this disease, under conditions of moderate infection, as well as would low-lime Bordeaux mixture. From tests made at the U. S. Pecan Field Station, Robson, La., in 1946, Ralph H. Sharpe reports[2] that zinc dimethyldithiocarbamate (Zerlate) controlled pecan scab on the Schley and Pabst varieties, with a smaller build-up in the black pecan aphis (*Melanocallis caryaefoliae* (Davis)) population, as well as did low-lime Bordeaux mixture.

In 1947, tests using 2–100 Zerlate in comparison with Bordeaux mixture, each of them with and without Dupont spreader-sticker, were made by the writer to control scab on the Schley variety at Albany, Ga. The spray materials were applied as follows:

First application: When the first leaves formed were half grown and before pollination, 2–100 Zerlate or 4–1–100 Bordeaux mixture was applied.

Second application: An application of 2–100 Zerlate or 6–2–100 Bordeaux mixture was made soon after pollination was complete, or when the tips of the small nuts had turned brown.

Third and fourth applications: The third spray application was made about 3 to 4 weeks after the second, and the fourth about 3 to 4 weeks after the third, 2–100 Zerlate or 6–2–100 Bordeaux mixture being used for each.

The results of these spray tests are given in table 1.

TABLE 1.—*A comparison of one year's spraying results with Zerlate and with Bordeaux mixture to control pecan scab. Schley variety planted 10 trees per acre. Albany, Georgia, 1947*

Treatment	No. of trees per test	Yield in lb. per tree	No. nuts per lb.	Percentage of kernel in the nuts
4 applications, Zerlate 2–100	9	34	55	63
4 applications, Zerlate 2–100 plus 1 qt. Dupont spreader sticker	9	30	57	61
1 application 4–1–100 Bordeaux mixture followed by 3 applications 6–2–100 Bordeaux mixture	9	40	60	60
1 application 4–1–100 Bordeaux mixture followed by 3 applications of 6–2–100 Bordeaux mixture plus 1 qt. Dupont spreader-sticker	9	32	63	57
Check, no spray	9	1	88	51

[1] Cole, John R. A comparison of home-made Bordeaux mixture with other fungicides for control of scab on the Schley and Moore varieties of pecans. Phytopath. **38**: 106–109. 1948.

[2] Correspondence with Ralph H. Sharpe, formerly assistant pomologist, U. S. Pecan Field Station, Robson, Louisiana; also personal observation by the writer.

Both Bordeaux mixture and Zerlate controlled the scab disease (Fig. 1). Although the trees sprayed with Bordeaux mixture produced the greatest number of pounds of nuts, the Zerlate-sprayed trees produced the heaviest individual nuts, as well as the highest percentage of kernel in the nuts. This would indicate that the Bordeaux-sprayed trees had a heavier set of nuts to begin with than those that were sprayed with Zerlate, since there was no evidence that early applications of the Zerlate spray were injurious to the pistillate flowers. All treatments gave outstandingly higher yields of nuts per tree as well as higher percentages of kernel in the nuts than were obtained from the unsprayed trees. Likewise, the yield of nuts per tree was reduced and the nuts were somewhat lighter in the treatments in

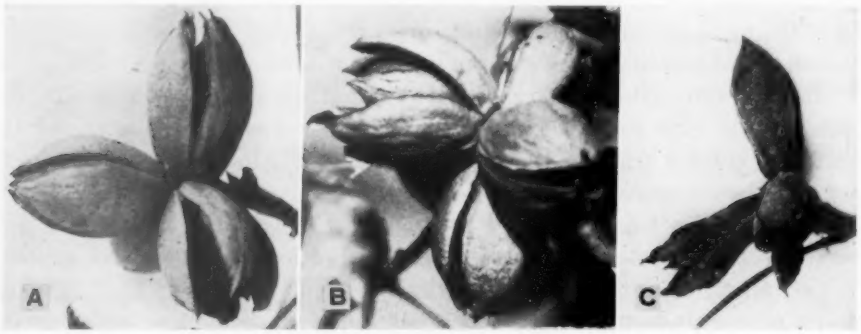


FIG. 1. Clusters of Schley pecan nuts. A. Received 4 applications of 2–100 Zerlate and are free of scab and of No. 1 quality. B. Received one prepollination spray of 4–1–100 Bordeaux mixture and 3 applications of 6–2–100 Bordeaux mixture. Free of scab and of No. 1 quality. C. Received no spray. Scab has made these nuts unmarketable. All photographed in October, 1947.

which the spreader-sticker was used, which indicates that this material may have been detrimental.

The foliage was a healthier green on the Zerlate-sprayed trees during the late summer and early fall than on either the Bordeaux-sprayed trees or the unsprayed checks. This color may have been caused by the absorption of zinc which is an ingredient of Zerlate, although the trees have not shown any visible signs of rosette, a zinc deficiency disease.

The infestation of black aphis was not a serious factor in this spray test, even on the Bordeaux-sprayed trees. There were fewer aphids on trees that were sprayed with Zerlate, however, than on those that were sprayed with Bordeaux mixture. There was a greater build-up of the black aphis population on both the Bordeaux-sprayed and the Zerlate-sprayed trees than there was on the unsprayed checks.—JOHN R. COLE, U. S. Department of Agriculture, Pecan Field Laboratory, Albany, Georgia.

Sori of Urocystis gladioli on Gladiolus Corm Scales.—Previously published illustrations of the smut disease of gladiolus, *Urocystis gladioli* W. G. Smith, are confined to drawings and a photograph of the spore balls. This

name has been cited so generally as *Urocystis gladioli* (Req.) W. G. Smith that attention should be called to Mason's explanation[1] that the correct form is *Urocystis gladioli* W. G. Smith. Ramsbottom[2] accepts Mason's citation, and Linder[3] cites it in the same manner. Linder also offers evidence that Requien's fungus, *Uredo gladioli*, is a synonym of *Puccinia gladioli* Cast.

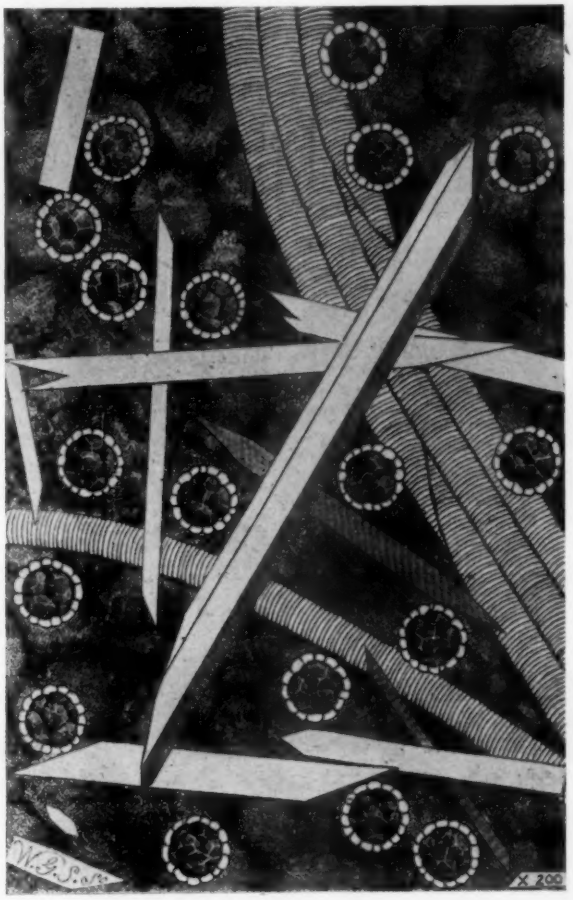


FIG. 1. Fragment of diseased corm showing decomposed cells, spiral vessels, crystals, and compound fungus spores, *Urocystis gladioli*, ×200. (After Smith[4, 5].)

The first illustration of *Urocystis gladioli* appeared in 1876 in the Gardener's Chronicle[4] with the original description of the species. Similar figures were also published by Smith[5] in the Monthly Microscopical Journal in the same year. Figures of the spore balls by Cooke[6] were later published

[1] Mason, E. W. New species and old. Trans. Brit. Mycol. Soc. **25**: 433–434. 1942.
[2] Ramsbottom, J. Conclusions and prospects. Trans. Brit. Mycol. Soc. **25**: 436–439. 1942.
[3] Linder, David H. Note. Mycologia **34**: 398–399. 1942.
[4] Smith, W. G. The gladiolus disease. Gard. Chron. (n.s.) **6**: 420–422, 2 figs. 1876.
[5] Smith, W. G. The gladiolus disease. Monthly Microsc. Jour. **16**: 304–311, pl. 163, 164 a, b. 1876.
[6] Cooke, M. C. Pests of the flower garden. Jour. Roy. Hort. Soc. **27**: 369–406, pl. 5, fig. 94. 1902.

in the Journal of the Royal Horticultural Society, and more recently a photograph by Hotson appeared in Mycologia.[7] These papers do not include an illustration of sori on the host, although Smith's figure 84, a

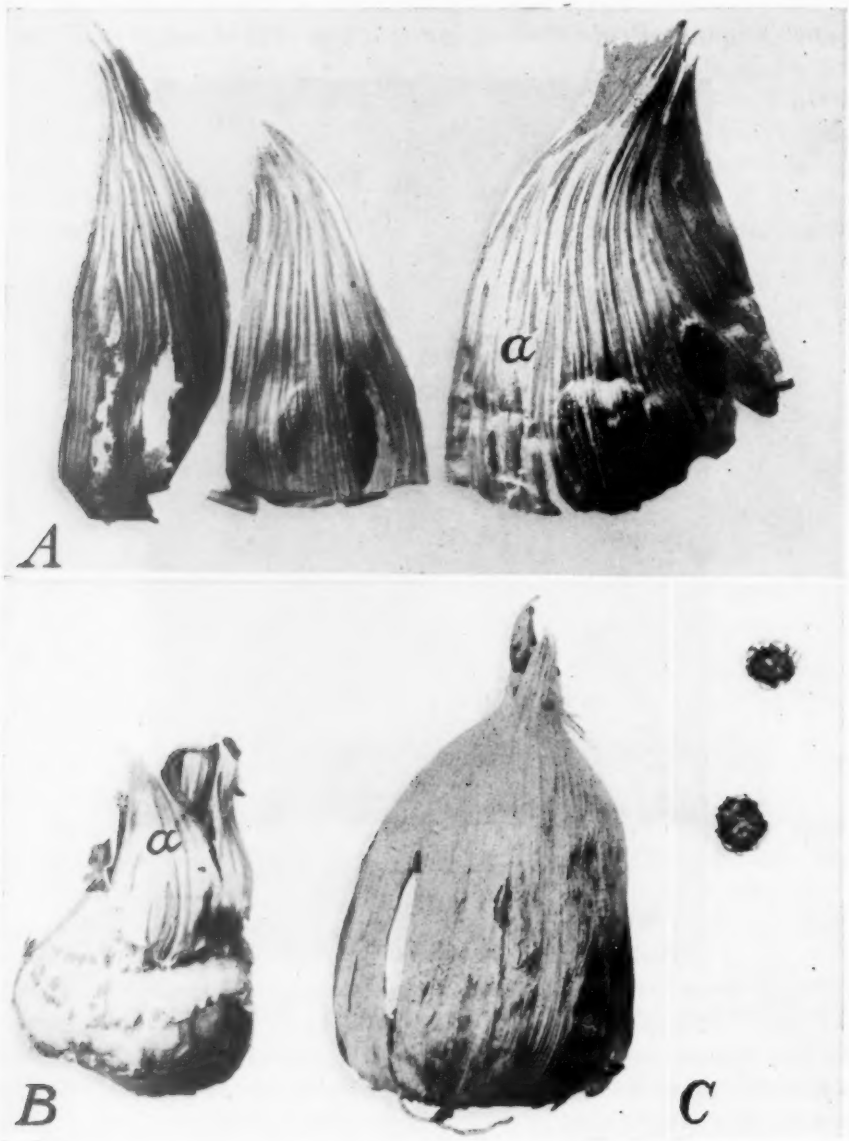


FIG. 2. *A*. Gladiolus corm scales bearing sori of *Urocystis gladioli*, ×2 1/2. *B*. Two gladiolus corms bearing smut sori, ×2. *C*. Spore balls of *U. gladioli*, ×580.

photograph of which appears here as figure 1, shows scattered spore balls on the host tissue.

[7] Hotson, H. H. The morphological distinction between *Urocystis gladioli* and *Papulaspora gladioli*. Mycologia 34: 52–58, fig. 1, 2. 1942.

Smith stated that there was a difference of opinion as to the injury caused by this smut, but van Poeteren[8] reported that in 1924 it was the cause of a serious disease of gladiolus in The Netherlands. It seems desirable, therefore, that American horticulturists and plant pathologists should become acquainted with the appearance of this smut in order that an introduction of the disease into the United States might be promptly recognized.

The photographs in figure 2 are of infected corms of gladiolus found in a shipment from Hillegom, Holland, at the Bureau of Entomology and Plant Quarantine inspection house at Hoboken, New Jersey, by P. E. Grayson on April 15, 1947.

The unbroken sori are roughly oval with the longer axis parallel with the nerves of the scale except when several sori become confluent (Fig. 2, *A* and *B*). The surface is roughened in parallel ridges which appear to be metamorphosed scale nerves. The grayish, lead color of the sori is little different from that of the healthy scale. Several sori in the figures appear too dark, but others are shown correctly (Fig. 2, *a* in *A* and *B*). The sori are usually raised slightly above the surface of the scale as low blisters. Those found on this material measure 4.5–6.5 by 1–5 mm., and are about 0.5 mm. thick. Two spore balls and a part of a third are shown in figure 2, *C*.

Grateful acknowledgment is made to John A. Stevenson, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, for advice in regard to the taxonomy of this fungus; to W. H. Wheeler, of the Bureau of Entomology and Plant Quarantine, for the photograph of Smith's figure and for assistance in obtaining bibliographical material; and to the Gardener's Chronicle Limited for permission to reproduce Smith's figure.—DONALD P. LIMBER, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Hoboken, New Jersey.

Cases of Scab on Violet and Pansy in Maryland.[1]—The extreme destructiveness of violet scab (*Sphaceloma violae* Jenkins) on cultivated and wild violets has become of greater moment as more and more instances of harm or loss from its attack have become known.[2] Transfer of affected plants

[8] Poeteren, N. van. Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het Jaar 1924. Verslag. en Meded. Plantenziektenkund. Dienst Wageningen No. 41, 62 pp. 1925.

[1] Scientific Article No. A 197. Contribution No. 2111 of the Maryland Agricultural Experiment Station.

This is an elaboration of our unpublished note "A case of violet scab on pansy," presented (by Jehle) at the meeting of the Potomac Division, American Phytopathological Society, Bureau of Plant Industry Station, Beltsville, Maryland, on Feb. 11, 1948. Thanks are extended to Dr. W. F. Jeffers and to Mr. R. L. Taylor who made the photographs as acknowledged in figure 1.

[2] Noble, R. J. Notes on plant diseases recorded in New South Wales for the year ending 30th June, 1938. Internat. Jour. Plant Protect. **13**: 25M–26M. 1939.

Smith, C. E., and A. G. Plakidas. Plant pests and problems. Home gardening for the South **6**: 491. 1946.

Thaxter, K. Scab in a Connecticut violet collection with notes of varietal reaction. U. S. Dept. Agr., Plant Dis. Reptr. **29**: 410–411. 1945.

Whetzel, H. H. The scab disease of violets. U. S. Dept. Agr., Plant Dis. Reptr. **28**: 769–770. 1944.

without knowledge that they were diseased doubtless has been an important factor in spreading the pathogen from locality to locality. Fortunately the disease is still absent in many places within its reported range in the United States.[3, 4] In the region of the District of Columbia, however, whence comes the earliest available record (1897–1899),[3] it is more or less widely distributed.[3, 5]

In summarizing the distribution of the disease in Maryland, Walker *et al.*[6] referred to an occurrence in 1936 on cultivated violets in a small commercial greenhouse in Maryland. This had come to the attention of Dr. Mark Woods, then of the University of Maryland, soon after the original publication by Massey and Jenkins,[7] on the disease. Because of this, Dr. Woods requested that Jenkins verify his diagnosis.

Further data relative to this case of violet scab are as follows: In autumn, 1935, plants of the varieties "Freys Fragrant" and "Princess of Wales" were purchased from a northern violet grower. They were full grown and diseased when received. When advice was sought several months later, most of the plants were practically ruined. A few of the less severely infected ones were removed from the greenhouse and planted outside.

These details were furnished recently by the grower[8] who again requested advice of the University of Maryland on a disease that was ravaging her pansy seedlings in autumn, 1947. Jehle visited the premises October 14.

The variety of pansy being grown was "Oregon Giant." The seedlings, from home-grown seed sown on August 3, occupied one and a half frames (3 × 6 ft.). Seedlings on the more elevated ground at the edge of the frames became diseased first and had been removed several weeks before Jehle's visit. By this time, practically all plants in the frames were affected and many were severely stunted (Fig. 1, A–C; D, *a* and *b*, and E). The disease was clearly violet scab.

Her two pansy beds had been "disease ridden" in the spring of 1947 the grower told us. Before transplanting she had noticed that a few seedlings in the frames were unhealthy. She recalled, too, that about five years ago, when she was growing pansies under glass, this same trouble had been present. In earlier years, happily, this pansy grower appears to have been spared losses resulting from this cause.

[3] Jenkins, A. E. "Oedema" or "wart" of cultivated violet identified as scab. Jour. Wash. Acad. Sci. **34**: 352–357. 1944.

[4] Jenkins, A. E., and A. A. Bitancourt. Spot anthracnoses in the United States and some island possessions. U. S. Dept. Agr., Plant Dis. Reptr. **31**: 114–117. 1947.

[5] Jenkins, A. E. Unusual collections of destructive fungi on plaintain and violet in the District of Columbia area. U. S. Dept. Agr., Plant Dis. Reptr. **24**: 370–372. 1940.

[6] Walker, E. A., R. A. Jehle, and A. E. Jenkins. Violet scab widely distributed in Maryland. U. S. Dept. Agr., Plant Dis. Reptr. **30**: 471–474. 1946.

[7] Massey, L. M., and A. E. Jenkins. Scab of violet caused by *Sphaceloma*. Cornell Univ. Agr. Exp. Sta. Mem. 170, 9 p., 1935.

[8] Miss Sophia Grodzicka, to whom acknowledgment is made for her painstaking cooperation in furnishing these and other facts pertaining to her violet and pansy culture since 1928.

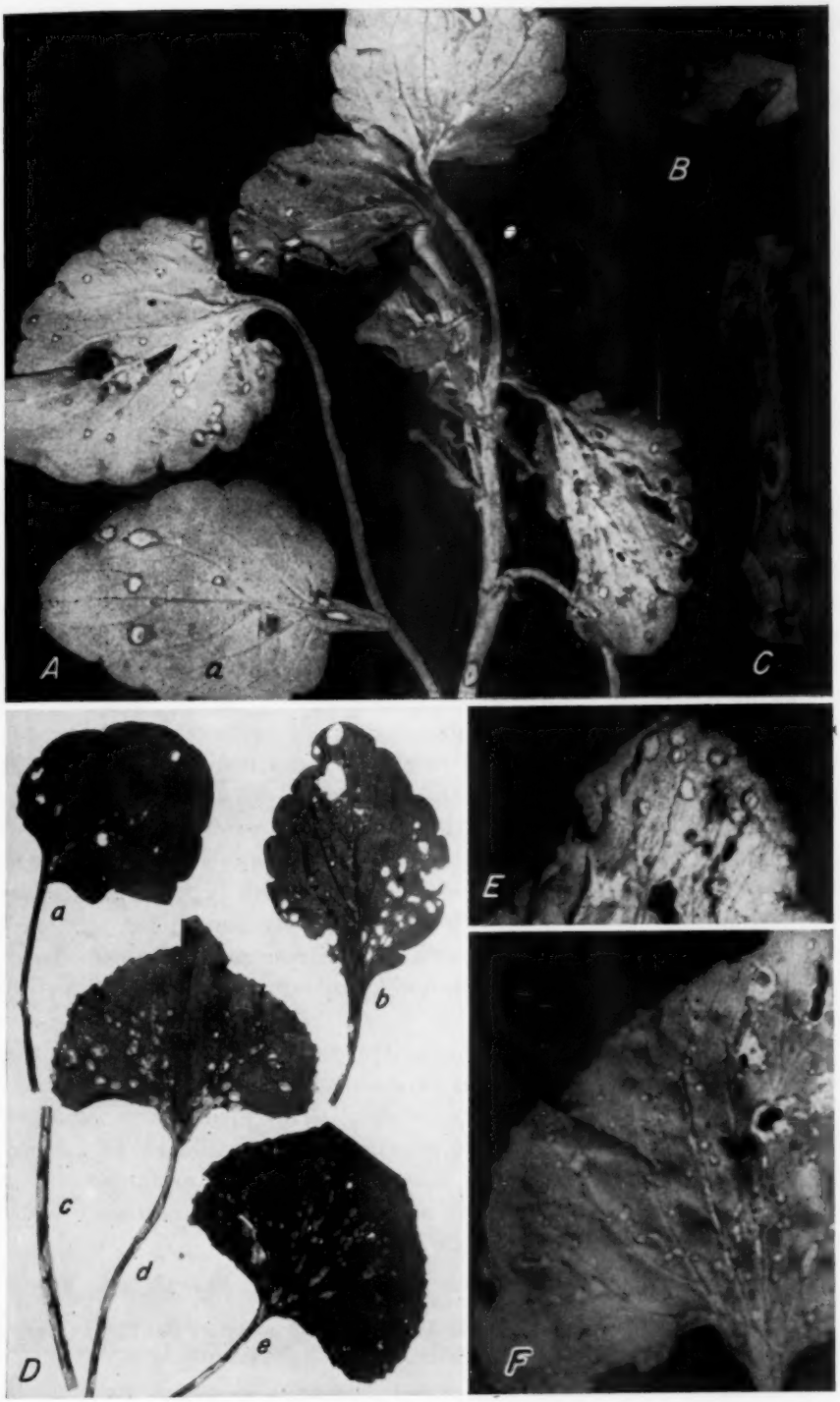


FIG. 1. A–C, D, *a* and *b*, and E. Growth of pansy seedlings affected by pansy scab, leaf surfaces in A, chiefly lower; D, *a*, upper and D, *b*, lower leaf surface. A–C and E, ×2; D, *a* and *b*, ×1; E, ×2.

D, *c*, *d*, *e*, and F. Scab on petioles (D, *c*, *d*, *e*), and lower (D, *d* and F) and upper (D, *e*) leaf surfaces of wild violets growing on same property as the pansies. D, *c–e*, ×1; F, ×2; D, photograph of dry pressed specimens by R. L. Taylor, the others of fresh specimens by W. F. Jeffers.

The scab fungus could well have been transferred to her pansies from affected violets on the premises. Last autumn (1947) wild violet volunteers growing near the pansies, along paths leading to them, and elsewhere on the premises were abundantly diseased (Fig. 1, D, *c–e*, and F).

In their inoculation experiments more than a decade ago, Massey and Jenkins (*loc. cit.*) proved the susceptibility of pansy as well as cultivated and wild violets. No data were available on the natural occurrence of the disease on pansy, but a few instances have since been reported.[9] The recent severe cases of scab of pansy and violet growing in close proximity obviously afford implications of economic significance.—R. A. JEHLE, University of Maryland, College Park, Md. and ANNA E. JENKINS, U. S. Department of Agriculture, Beltsville, Md.

A Convenient Method for Isolating Slow-growing Pathogenic Fungi from Plant Tissues.—While studying the scab of camellias, a method was employed which proved to be very useful and convenient for isolating not only the fungus causing camellia scab but also for other slow-growing pathogenic fungi on other hosts (Table 1). The writer does not claim originality for this method but believes that it should be more generally used in pathological work. It represents a modification of the method described by Bitancourt[1] which was originally brought to the plant pathology laboratory of Louisiana State University by his colleague, S. C. Arruda,[2] while doing graduate work. As originally described by Bitancourt, the method consisted of cutting free hand (approx. $0.5 \times 0.3 \times 0.02$ mm.) sections of plant tissue through the lesion, or, more specifically, through the fruiting body of the pathogen with the aid of a dissecting microscope, and transferring these to agar drops on glass slides kept in humid atmosphere in Petri dishes. After a suitable period (24–48 hours) the sections on the agar drops were examined with the microscope, and those showing growth of what appeared to be *Sphaceloma* were transferred to plates or tubes.

The modification made by the writer consisted of placing the tissue sections on hanging drops of agar on cover slips suspended on glass rings in a moist chamber, instead of on slides.[3] This modification eliminates, to some extent, the danger of contamination as it provides a separate sealed chamber for each agar drop. A tissue section can be examined with the microscope through the glass cover slip as long as seems necessary without the risk of contamination from the air.

[9] Jenkins, A. E. Additional records of violet scab. U. S. Dept. Agr., Plant Dis. Reptr. **22**: 86–88. 1938. Cf. also Whetzel, footnote 2.

[1] Bitancourt, A. A., and Anna E. Jenkins. Ciclo evolutivo de *Elsinoe australis* Bitancourt & Jenkins, agente da verrugose da laranja doce. Arq. Inst. Biol. S. Paulo 10: 129–146. 1939.

[2] Arruda, S. C. Sphaceloma diseases of camellia and other plants. Thesis, deposited in library, Louisiana State University. 1942.

[3] Hanna, W. F. A simple apparatus for isolating single spores. Phytopath. **18**: 1017–1021. 1928.

Seven or eight holes are cut in two layers of filter paper of a size to fit the bottom of a 15-mm. Petri dish. Glass rings are placed over the holes and cover slips on the glass rings. The dishes thus prepared are sterilized dry in an autoclave. After the dishes have cooled, drops of melted agar are placed on the cover slips and enough sterile water poured into the dish to wet the filter paper. It is essential to wet the filter paper almost immediately after the agar drops have been deposited on the cover slips in order to prevent drying of the agar during the subsequent operations. Carrot agar[4] has been commonly used, but other media can be used. Bitancourt and Arruda used potato-dextrose agar, and the writer has also used potato-dextrose and oatmeal agars with success.

TABLE 1.—*Examples of successful isolation of slow-growing fungus pathogens by placing thin sections of diseased plant parts on hanging agar drops*

Kind of material	No. of sections plated	Pure cultures of desired pathogen	Various other fungi	Bacteria	No growth
A. Camellia scab (*Sphaceloma* sp.)					
1. Black spot type	20	6	7	0	7
2. Angular spot type	37	16	9	1	11
3. Zonate spot type	21	12	6	0	3
4. Corky excrescence	215	52	75	3	85
B. Chinese holly scab (*Sphaceloma* sp.)					
1. Black spot on leaves	83	8	19	0	56
2. Old leaf distorting lesion	20	6	9	0	5
3. Young do	35	12	9	0	14
4. Lesions on shoots	49	23	22	1	3
5. Lesions on berries	40	9	18	0	13
C. Dewberry anthracnose (*Elsinoe veneta*), lesions on canes	40	6	3	1	30
D. Strawberry scorch (*Diplocarpon earliana*)	51	30	7	4	10
E. Strawberry leaf-spot (*Mycosphaerella fragariae*)	49	8	0	1	40
Total	660	188	184	11	277

Materials (leaves, stems, berries) were washed thoroughly with a brush in running water, dipped in calcium hypochlorite solution for 1 to 2 minutes, and, without rinsing, dried between layers of sterile filter paper. Then a small piece containing a lesion was placed in pith and clamped tightly in a screw clamp. With relatively rigid material, like leaves of camellia or holly, the piece to be sectioned was often allowed to project 1 to 2 mm. above the pith thus lessening the chances of contamination from contact with the pith. Then, by holding the clamp with the left hand under the dissecting microscope, small slices of tissue (about 0.5 mm. × 25 μ) were sectioned with a sharp razor blade and transferred by means of the transfer needle to the agar drops. The cover slips were then lifted

[4] Approximately 300 gm. sliced carrots per liter of water, autoclaved for 20 minutes, strained through cotton, +20 gm. agar + 20 gm. sucrose.

with the forceps and reversed so that the agar drops hung within the glass ring. Precautions usually were taken to lessen the chances of contamination by flaming the blade lightly before cutting the section and also the needle before transferring the section to the agar drop. Actually, these precautions did not appear to be necessary. In several instances, the material to be sectioned was washed but not surface-disinfected, and in others not even washed, yet practically no bacterial contaminations occurred. Apparently the small size of tissue used greatly minimized the probability of a bacterial cell being lodged on it.

It was possible to examine the tissue pieces on the hanging drops with the microscope without exposing them to contamination and the development of the colonies was followed. By adding sterile water to the dish to prevent drying, the cultures were kept as long as desired. Usually, 48 hours were sufficient for *Sphaceloma* colonies to be recognizable and ready for transfer.—A. G. PLAKIDAS, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Local Lesions with Potato Virus Y.—In an attempt to find a satisfactory local lesion host for potato virus Y (*Marmor upsilon* Holmes),[1] over a hundred plant species were tested for their reaction to mechanical inoculation with this virus. In general the plants were used after the older leaves had attained maximum size but before flowering had begun. The leaves were dusted lightly with 400-mesh carborundum and then rubbed with a gauze pad saturated with freshly expressed juice from a diseased *Nicotiana glutinosa* L. plant. Four new local lesion hosts were found, *Chenopodium urbicum* L., *Physalis floridana* Rydb.,[2] *Lycium halimifolium* Mill., and *L. chinense* Mill. There is some doubt, however, as to whether or not the two species of *Lycium* are distinct from the *L. barbarum* reported by Dennis[3] as a local lesion test plant for virus Y.

Pale green to whitish-yellow non-necrotic lesions appear on the inoculated leaves of *Chenopodium urbicum* 14–16 days following inoculation (Fig. 1, A). Distinct brown necrotic lesions develop on inoculated leaves of the Lycium species in about 10 days (Fig. 1, B). Infection is restricted to the inoculated leaves in both Lycium species and in *C. urbicum*. Small necrotic spots appear on inoculated leaves of *Physalis floridana* about 9–10 days following inoculation (Fig. 1, C). The lesions enlarge and finally coalesce. At this stage the leaves show epinasty and begin to abscise acropetally. Concurrently, systemic symptoms appear, consisting of rugosity and necrotic spotting of the young leaves followed by leaf abscission. Lesions on inoculated leaves can be counted by about the 12th day.

Vigna sinensis Endl.[4] and certain potato (*Solanum tuberosum* L.) varie-

[1] Potato veinbanding strain. Kindly supplied by E. S. Schultz.
[2] Identified by Dr. W. C. Muenscher.
[3] Dennis, R. W. G. A new test plant for potato virus Y. Nature 142: 154. 1938.
[4] Chester, K. S. Serological evidence in plant virus classification. Phytopath. 25: 686–701. 1935.

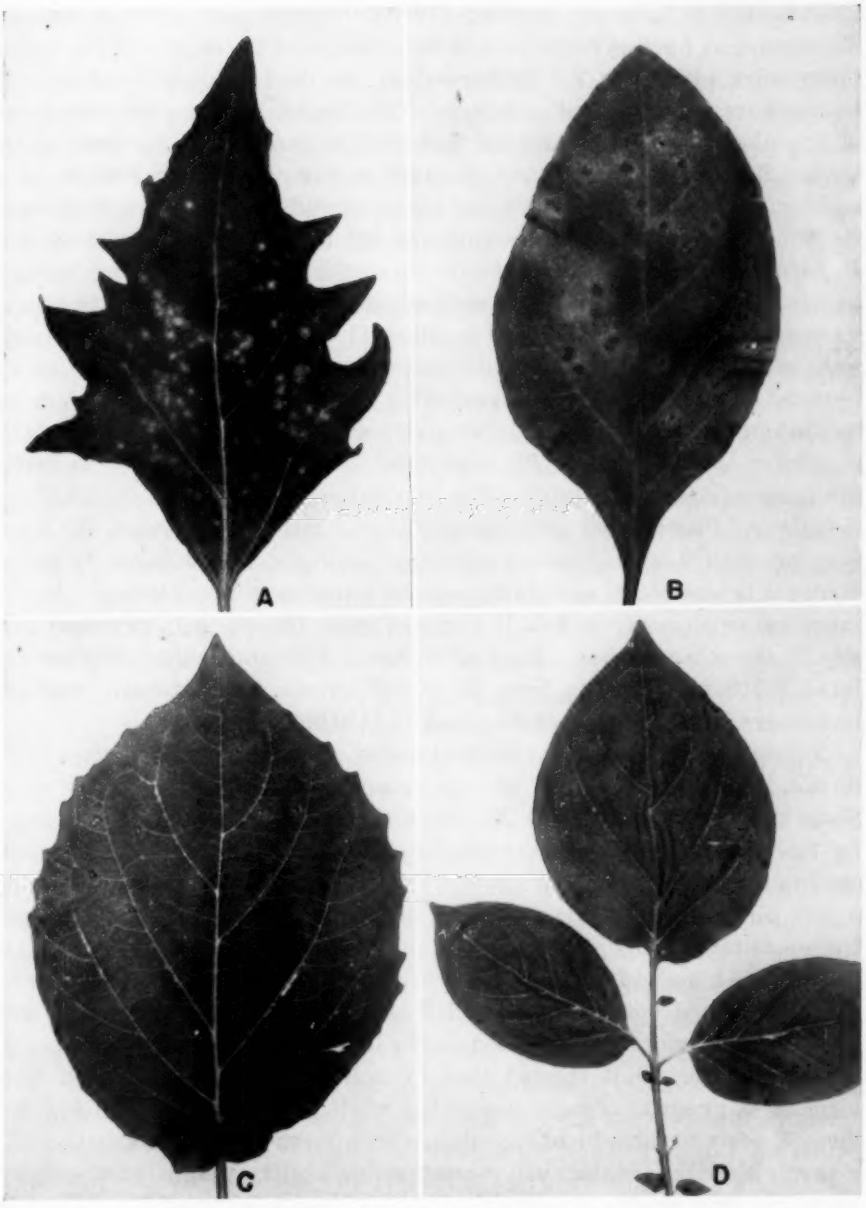


FIG. 1. Local lesions on leaves inoculated with potato virus Y. A. *Chenopodium urbicum* (×1). B. *Lycium halimifolium* (×¾). C. *Physalis floridana* (×½). D. U.S.D.A. potato seedling 41956 (×¼).

ties[5] and seedlings[6] have been reported as reacting to mechanical inoculation with potato virus Y by the formation of local lesions. In further tests, *V.*

[5] Dykstra, T. P. A study of viruses infecting European and American varieties of the potato Solanum tuberosum. Phytopath. 29: 40–67. 1939.

[6] Hutton, E. M., and J. G. Bald. The relationship between necrosis and resistance to virus Y in the potato. 1. Greenhouse results. Jour. Coun. Sci. Indus. Res. (Aust.) 18: 48–52. 1945.

sinensis, U.S.D.A. potato seedling 41956,[7] *Chenopodium urbicum*, *Physalis floridana*, and *Lycium halimifolium* were compared as test plants for quantitative work with virus Y. Carborundum was used in all inoculations. No lesions were obtained on *V. sinensis*. The dilution curves obtained on each of the other test plants indicated that all four can be used for quantitative work. *L. halimifolium* was not included in subsequent tests because of its undesirable growth habits. Virus solutions differing in concentration by 20 per cent generally gave a significant difference in number of lesions on *P. floridana*, *C. urbicum*, and the potato seedling when 48 or more leaves or half-leaves were inoculated with each solution. Modified half-leaf techniques were used with *P. floridana* and seedling 41956 and a randomized arrangement of the inoculated leaves with the other species. In these tests juice of diseased *N. glutinosa* plants was diluted 1–100 or 1–200 when *P. floridana* was inoculated and 1–25 or 1–50 when either of the other two was inoculated.

Under most conditions *Physalis floridana* has proved to be the best of the local lesion plants tested. The leaves are well suited for the half-leaf technique. Plants react satisfactorily over a rather long period, beginning just prior to flowering and continuing until the leaves begin to yellow. Hence it is possible to use plants with as many as 16 large leaves. A given inoculum produces from 4 to 10 times as many lesions on *P. floridana* as on any of the other species. Juice of diseased *Nicotiana glutinosa* plants diluted 1–100 will produce from 20 to 200 lesions per half-leaf. Scattered lesions are not infrequent at dilutions of 1–10,000.

Physalis floridana cannot be used under all conditions. Necrotic lesions do not develop satisfactorily when the plants are held near 80° F. or above. Some strains of potato virus X (*Annulus dubius* Holmes) produce lesions on this species (also on *Chenopodium urbicum* and the Lycium species) similar to those caused by virus Y. Preliminary tests with potato seedling 41956, which is immune from virus X, indicate that it may be useful not only for quantitative work with naturally infected potatoes (where virus X may be present) but also for indexing work. Juice of triturated shoots or sprouts from Y-infected potatoes, diluted 1–5 or 1–10, regularly caused the formation of many lesions when rubbed on the leaves of this seedling.

Other experiments showed that *Physalis floridana* can be used where virus X is present. Plants inoculated when young with a mild strain of virus X react to subsequent inoculation with virus Y by the formation of as many or more lesions than will corresponding healthy plants inoculated with virus Y. Mild strains of virus X produce no marked symptoms and protect the plant against all tested strains of virus X.—A. FRANK ROSS, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Longevity of Fungus Cultures under Mineral Oil.—In 1946 the senior author[1] reported on the two-year survival of *Diplodia zeae*, *Gibberella zeae*,

[7] Found to produce necrotic lesions following mechanical inoculation with virus Y (Fig. 1, D) by Dr. W. F. Mai. Personal communication, 1946.

[1] Wernham, C. C. Mineral oil as a fungus culture preservative. Mycologia 38: 691–692. 1946.

Helminthosporium maydis, and *Nigrospora oryzae* placed under mineral oil March 15, 1944.

Since the appearance of this brief note Buell and Weston[2] have reviewed the pertinent literature and have presented considerable data pertaining to the preservation of a number of fungi on media submerged under mineral oil. In addition Edwards, Buell, and Weston[3] have published data from which they deduce that the prolonged life, without transfer, of *Sordaria fimicola* under mineral oil is due to a reduction in oxygen consumption.

Although Henry[4] has reported good growth with unchanged morphology of seventeen wine yeasts held seven years under mineral oil, data on other fungi are not available for periods longer than three and one-half years. Little, too, is known about preservation of pathogenicity.

On March 15, 1948, four years after treating them with mineral oil, the four maize pathogens[1] were transferred to standard Difco potato-dextrose agar. The *Diplodia* culture had been used as a stock culture so frequently that only portions of the agar substrate could be transferred. Mycelium appeared to be wholly depleted: the other three cultures still yielded plenty of hyphal material for transfer.

Within three days luxuriant growth of all but *Diplodia* had taken place. Within nine days two of four *Diplodia* transfers gave good cultures but the remaining two did not grow. These subcultures definitely establish a length of survival of four years.

During the 1947 season *Diplodia, Gibberella,* and *Helminthosporium maydis* were tested for pathogenicity. All appeared to be actively pathogenic after three years under oil.

Following the appearance of the original note in Mycologia, Dr. W. R. Goss, in correspondence, pointed out that he had used the mineral oil method but had found it unsatisfactory for pathogenic Phycomycetes held at room temperature.

The junior author investigated the behavior of *Phytophthora infestans* which is used in this laboratory as a standard for testing fungicides. Forty-five cultures on Lima-bean agar were treated and held at 12° C. At the end of one year, only two were nonviable. Twenty-eight of the forty-three remaining ones were subcultured, and the twenty-eight original as well as the subculture tubes were held under mineral oil for an additional year. Twenty of the two-year tube cultures survived; all of the one-year subcultures survived. Pathogenicity of the two-year survivors was reduced but not lost when tested on potatoes grown in the greenhouse.

[2] Buell, C. B. and Wm. H. Weston. Application of the mineral oil conservation method to maintaining collections of fungus cultures. Amer. Jour. Bot. **34**: 555–561. 1947.

[3] Edwards, G. A., Caroline B. Buell, and Wm. H. Weston. The influence of mineral oil upon the oxygen consumption of *Sordaria fimicola*. Amer. Jour. Bot. **34**: 551–555. 1947.

[4] Henry, B. S. The viability of yeast cultures preserved under mineral oil. Jour. Bact. **54**: 264. 1947.

Sclerotinia fructicola and *Venturia inaequalis* have also been kept satisfactorily for two years without transfer but no test was made of pathogenicity.—C. C. WERNHAM and H. J. MILLER, Department of Botany, The Pennsylvania State College.

Curly Top of Muskmelon.—Severin[1] reported finding muskmelon naturally infected with curly-top virus during 1925 and 1928. The disease has been destructive to the commercial crop in the west central and southern portions of the San Joaquin Valley of California during some seasons. It was also reported serious on the early plantings in the Salt River Valley area of Arizona during the 1947 season.

Diseased plants in the cotyledon and the one or two-leaf stage (Fig. 1, A)

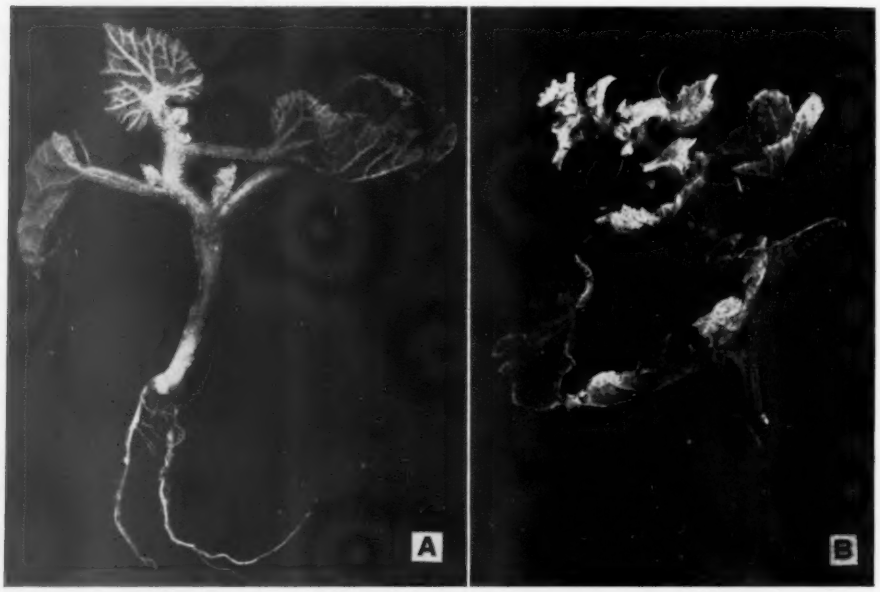


FIG. 1 A. Curly-top diseased muskmelon plant from Arizona, with distorted leaves and prominent veins. B. Portion of a Persian melon plant from a commercial field near Murray, California, showing dwarfing and distortion as a result of curly-top infection.

were sent by Orin A. Hills, Bureau of Entomology and Plant Quarantine, from Arizona about mid-April, 1947, and curly-top virus was recovered from them. Another shipment of plants from the same field was received about mid-June. These plants, with runners a foot or two long, were appreciably stunted and distorted, and curly-top virus was recovered from them. The melon commonly grown in this area of Arizona is known as the Mildew Resistant 45 and the plants tested were reported to be that variety. Diseased cantaloupe plants grown near Murray, California, were collected May 21, 1947, by Dr. Eubanks Carsner, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering. These

[1] Severin, H. H. P. Some host plants of curly top. Hilgardia 3: 339–384. 1928.

plants were infected by the curly-top virus and had runners one to two feet long. Plants of Persian melon from the same area were collected by Dr. Carsner August 13, 1947, and at the time were carrying mature melons although they were much dwarfed and distorted. Practically all plants in the field were reported as showing good curly-top symptoms (Figure 1 B).

A few greenhouse inoculations of young melon plants made using one or two leafhoppers per plant resulted in a comparatively small percentage of infection, while greater numbers of leafhoppers gave a high percentage of infection. These results, and the fact that several plants escaped infection with the heavier inoculations, indicate that muskmelon seedlings are distinctly more resistant to curly-top infection than are young sugar beet plants.

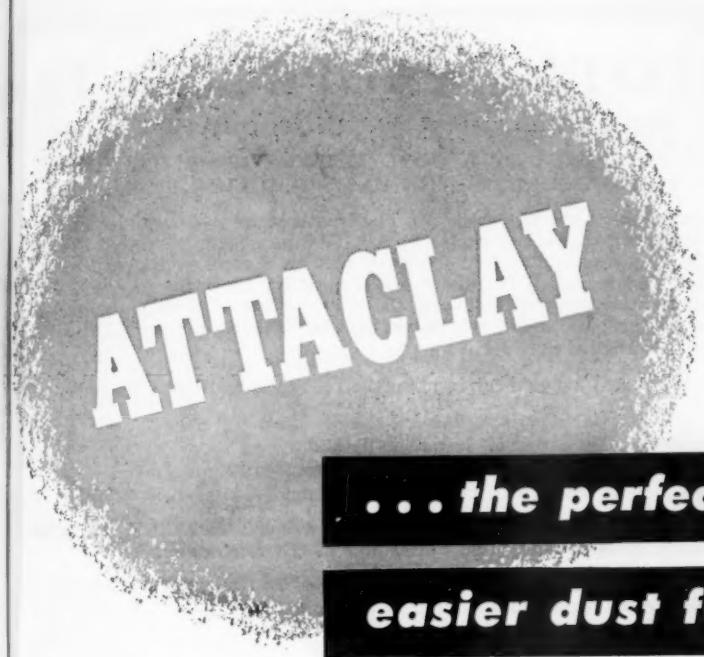
FIG. 2. Mildew Resistant 45 muskmelon variety. Two dwarfed plants are infected by curly-top virus strain 10 and large plant is not infected. Cotyledons on infected plants are light yellow. Photographed 23 days after inoculation.

In order to learn more concerning the susceptibility of muskmelon varieties to curly top, greenhouse inoculations were made on plants of the following varieties: Rocky Ford, Tip Top, Honey Rock, Hearts of Gold, Japanese melon, Honey Dew, Cranshaw, Golden Beauty Improved Casaba, Golden Hybrid Casaba, Persian, Hale's Best 45, and variety 45 from four other sources. All were started in 6-inch pots but a few were transferred to 10-inch pots to see the influence of greater opportunity for root growth. Most of the plants in each group were inoculated in the young cotyledon stage, using 15 to 20 leafhoppers to a group of three plants and a large

cage which covered all the plants. Some of each group were inoculated, using the small leaf cages with two or three leafhoppers to a cage and three or four cages on each plant. Infections were obtained equally well with both types of cages. Inoculations were made with virus strains 1, 2, 3, 4, 5, 6, 8, 9 and 10, as well as some selections of curly-top virus from the field. Infection was obtained with each of the virus strains except 2 and 4. Only plants of the Gem variety were inoculated with strain 4. Numerous attempts to recover curly-top virus from plants inoculated with strains 2 and 4 gave negative results. This would indicate that the less virulent strains 2 and 4 do not infect these melons.

Although infection was rather general among the melon varieties, the apparent amount of injury differed greatly. Ten days to two weeks after inoculation there was distinct yellowing of the cotyledons on many of the plants (Fig. 2) in the four lots designated as "mildew resistant 45", Hale's Best 45, Honey Rock, Rocky Ford, and Hearts of Gold. Three weeks after inoculation the four lots of Mildew Resistant 45, Hale's Best 45, and Honey Rock were easily grouped as the ones most severely injured, while the casabas and Cranshaw appeared to be least injured. A high percentage of the plants showing definite symptoms were severely dwarfed (Figure 2) and many of these died. Some infected plants lived for more than five months after inoculation and curly-top virus was readily obtained, five months after inoculation, from one dwarfed plant infected with strain 1. Most such plants died within two or three months. One plant infected with strain 10 virus grew quite normally and had a runner over ten feet long, but virus was readily obtained from it four months after inoculation. Nearly 200 tests were made from the different melon varieties to determine the presence of curly-top virus in them.

It is clearly evident that the curly-top virus may induce serious injury in commercial plantings of muskmelons, and serious losses were reported for certain areas in the western San Joaquin Valley of California and the Salt River Valley of Arizona during 1947. Some of the evidence secured in greenhouse experiments suggests the possibility that there may be opportunity for selection and breeding of muskmelon for resistance to curly-top.—N. J. GIDDINGS, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.



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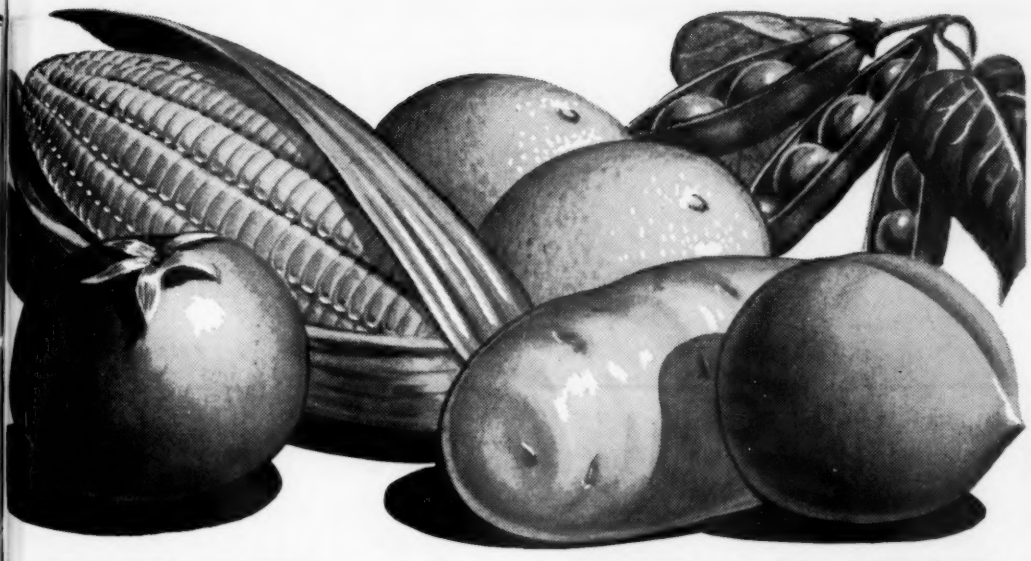

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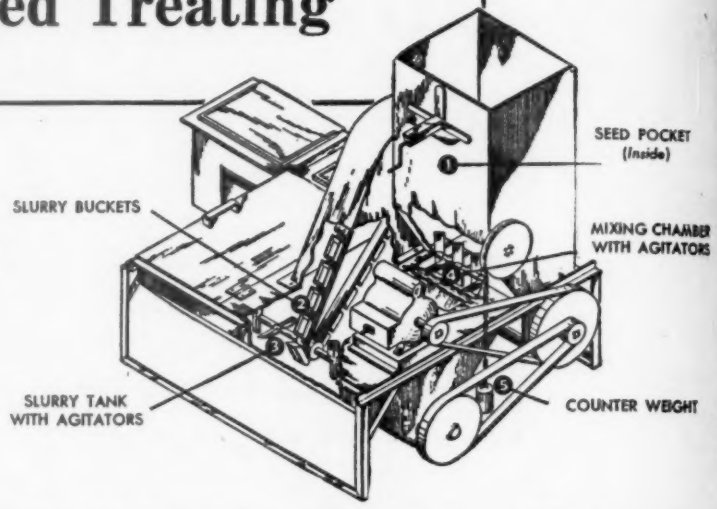

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